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JOURNAL OF THE FISHERIES RESEARCH BOARD OF CANADA

The *Journal of the Fisheries Research Board of Canada* is devoted to original research papers and notes concerning aquatic organisms or environments relevant to Canada. Review articles are also welcomed; they should be timely, critical appraisals of the state of knowledge in broad areas of research. Submissions, in either English or French, are accepted without limitations as to origin, but must not have been published elsewhere or copyrighted.

Guides to authors and typists are given in each issue of the *Journal*.

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Life Cycles of *Limnocalanus macrurus* and *Senecella calanoides*, and Seasonal Abundance and Vertical Distributions of Various Planktonic Copepods, in Parry Sound, Georgian Bay

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CARTER, JOHN C. H. 1969. Life cycles of *Limnocalanus macrurus* and *Senecella calanoides*, and seasonal abundance and vertical distributions of various planktonic copepods, in Parry Sound, Georgian Bay. J. Fish. Res. Bd. Canada 26: 2543–2560.

The life cycles and vertical distributions of *Limnocalanus macrurus* and *Senecella calanoides* and the seasonal abundance and vertical distributions of adults of other planktonic copepods in Parry Sound, Georgian Bay, were determined from diurnal vertical plankton hauls taken on 14 occasions from June 19, 1967, through November 11, 1968. The most abundant cyclopoid copepod on most occasions was *Cyclops bicuspidatus thomasi*. *Tropocyclops prasinus* was common, particularly in summer and autumn. *Mesocyclops edax* and *Cyclops vernalis* were scarce. *Cyclops bicuspidatus thomasi* and *T. prasinus* were usually concentrated in the upper strata, *T. prasinus* seldom being deeper than 3 m during summer.

The most abundant calanoid copepod was *Diaptomus ashlandi*. *Diaptomus minutus* and *D. oregonensis* were also common, but *D. sicilis* was rare. *Diaptomus ashlandi* and *D. oregonensis* occupied much the same depths in the metalimnion during the periods of thermal stratification but were deeper in spring and fall. *Diaptomus minutus* was concentrated mainly in the epilimnion even when thermal stratification was slight. *Epischura lacustris* and *Eurytemora affinis* were scarce. *Limnocalanus macrurus* was abundant and monocyclic, becoming adult in late spring but not spawning until autumn; older copepodites inhabited somewhat deeper strata than younger stages. *Senecella calanoides* was less abundant than *L. macrurus* but was also monocyclic, becoming adult in autumn and spawning almost immediately; all instars were usually found in deeper water than *L. macrurus*. The possibility that *S. calanoides*, because of its longer period of development, is better adapted to life in Parry Sound than *L. macrurus* is discussed.

Received March 24, 1969

INTRODUCTION

NUMEROUS STUDIES have been made on the zooplankton of the St. Lawrence Great Lakes (see Davis, 1966). However, excepting Sars' (1915) paper, few have included Georgian Bay. Also, few detailed investigations have been made on life histories of Great Lakes copepods, Davis' (1961) study of calanoid breeding cycles in Lake Erie being a notable exception. The present paper attempts to meet in some degree these two neglects with an outline of the variations in abundance at a series of depths of cyclopoid and small calanoid copepods and a detailed description of the life cycles of *Limnocalanus macrurus* and *Senecella calanoides* in Parry Sound, Georgian Bay, during a 17-month

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period. The life history of *S. calanoides* has nowhere been described previously; that of *L. macrurus* has been observed closely only in Ekoln, Sweden, by Ekman (1907).

Parry Sound (45°20′N, 80°05′W) is a deep basin midway along the eastern shore of Georgian Bay (Fig. 1). It has a total area of approximately 80 km² and is connected to Georgian Bay by a short channel of moderate depth about 800 m wide at the narrowest point. Maximum depth of the Sound is at least 113 m, slightly greater than given on the official Canadian Government charts. It is fairly oligotrophic as indeed is Lake Huron generally (Beeton, 1965). The water is neutral to slightly alkaline (*p*H 7.0–7.7) and is undoubtedly strongly influenced by the soft waters flowing in off the surrounding Precambrian Shield, mainly via the Seguin River. Because of its considerable depth Parry Sound probably contains most, if not all, planktonic copepod species found in Georgian Bay; its small area and favourable location allow convenient sampling at most times during the year.

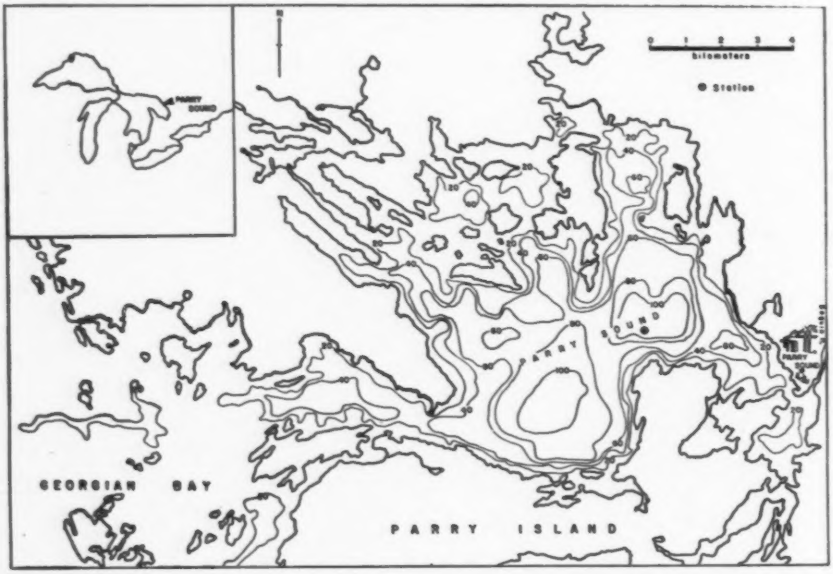

FIG. 1. Bathymetry of Parry Sound. Depth contours in meters. (Redrawn from Canadian Hydrographic Service Chart No. 2284, 1967.)

METHODS

A single station was established in the deepest part of Parry Sound where sounded depths ranged from 105 to 113 m (Fig. 1). Sampling was carried out between 1100 and 1530 hr on 14 occasions (Table 1) between June 19, 1967, and November 11, 1968. A chartered 28-ft launch was employed on all dates except March 9, 1968, when the Sound was covered by 45 cm of ice.

TABLE 1. *Limnocalanus macrurus* and *Senecella calanoides* in Parry Sound. Percentages of generations adult (C6) and of adult females with spermatophores, and sex ratios of older copepodites. Numbers in parentheses are numbers of specimens in samples of less than 100.

	% of generation C6		% ♀♀ with spermatophores		Sex ratio (♂/♀)				
					C4	C5		C6	
Date	*L. macrurus*	*S. calanoides*	*L. macrurus*	*S. calanoides*	*S. calanoides*	*L. macrurus*	*S. calanoides*	*L. macrurus*	*S. calanoides*
				1967					
June 19	45[a]	0	2	–	1.04	0.82(63)	1.67(8)	0.76	–
July 17	56[a]	0	1	–	1.24	0.89	0.45	1.01	–
Aug. 14	88	4	0	0(6)	1.50(40)	1.16(41)	1.07	0.87	1.0(12)
Oct. 3	99	18	11	3(17)	0.20(5)	2♂♂only	0.96	0.90	0.93(33)
Dec. 7	100	100	45(39)	14(13)	–	–	–	0.95(76)	1.38(31)
				1968					
Mar. 9	1[a]	1[a]	29(12)	25(4)	–	2.45(17)	–	1.08(25)	0.50(6)
Apr. 22	6[a]	0	2(53)	–	2.07(9)	0.98	–	0.85(98)	–
May 22	22[a]	0	2	–	1.87(43)	0.85	–	0.90	–
June 11	65[a]	1[a]	2	0(2)	0.83	1.01	0.20(5)	0.85	2 ♀♀ only
July 5	84	0	0	–	1.24	1.24(47)	0.95(70)	0.90	–
Aug. 2	97	1	0	–	1.67(80)	0.17(7)	1.18	0.92	2 ♂♂only
Aug. 29	99	3	2	0(4)	4.60(11)	–	1.10	0.92	2.5(10)
Sept. 23	100	21	16	5(31)	–	–	0.87	1.0	1.06(64)
Nov. 11	100	90	55	15	–	–	1.20(35)	1.13	1.23

[a]May include C6 of previous generation.

Sampling was done with conical nylon mesh nets hauled vertically by hand from 3 m above the bottom at a rate of approximately 0.5 m/sec. Larger copepods were taken in single hauls of a closing net of 0.5-m mouth diam, the mesh used from June 19 through October 3, 1967, being no. 6 (apertures 0.239 mm) and that on the remaining dates no. 10 (apertures 0.158 mm). Smaller forms, comprising all nauplii of *L. macrurus* on all dates, and adult *Tropocylops prasinus* when the closing net was of no. 6 mesh, were taken in two combined hauls of a nonclosing net of 30-cm mouth diam with no. 20 mesh (apertures 0.076 mm). Two lines were employed with the closing net: the first to lower it and then haul it through the water layer being sampled, and the second, attached midway along the length of the net, to pull it to the surface effectively closed with the mouth trailing downward.

In this way the water column was sampled in a series of conterminous strata of various depths. The usual series comprised five 3-m layers from the surface to 15 m, three 5-m layers from 15 to 30 m, six 10-m layers from 30 to 90 m, and a final layer from 90 m to 3 m above the bottom. However, from July 17 through October 3, 1967, the 3-m layers were extended down to 30 m and the 5-m layers to 50 m. On May 22, 1968, because of a slight miscalculation, 3-m layers were sampled from the surface to 12 m, 5-m layers from 12 to 27 m, 10-m layers from 27 to 87 m, and a final layer from 87 to 107 m.

Since the closing nets gave no evidence of clogging, they were assumed, for purposes of quantifying the data, to be 100% efficient although this can be an approximation at best. However, there was occasionally obvious clogging of the nonclosing net and therefore the smaller forms must be considered somewhat undersampled.

Copepods were counted in the laboratory under a dissecting microscope. Subsamples of 25 ml were extracted after the original samples had been thoroughly mixed. The number of subsamples removed from each sample varied according to concentration of copepods. From the closing net samples at least 75 adults of each species and 75 specimens of each copepodite stage of *L. macrurus* and *S. calanoides* were counted, or the total present when 75 were not available. All the nauplii of *S. calanoides* in the closing net samples were counted. Aliquots of 25 ml were also removed from the nonclosing net samples until a total of 150 *L. macrurus* nauplii, and 75 *T. prasinus* adults on the first four dates, were counted.

In determining seasonal abundance, totals of each species or instar from all depths on each date were expressed as numbers/m^2. This procedure is not valid in the strict sense since hauls were not taken below 3 m from the bottom. But the animals were usually concentrated at a few depths rather than spaced evenly throughout the water column, and the method obviates the unsuitable alternative of quantifying per unit volume. Besides, since the numbers of all species tapered off considerably toward the bottom, the error is probably minimal.

In determining vertical distribution, totals from each depth were expressed as numbers/m^3. Since only the larger forms were captured by the closing nets, vertical distribution of *L. macrurus* nauplii was not determined. Because no. 6 mesh is inefficient in capturing *T. prasinus* adults, vertical distribution of this species from June 19 through October 3, 1967, was not determined either.

The keys of Wilson and Yeatman (1959) were used for identification of adults. Copepodites and nauplii of *L. macrurus* were identified with Lindquist's (1959) descriptions of the closely related *Limnocalanus grimaldii*. The copepodites of *S. calanoides* were readily separable by numbers of legs and urosome segments, these being identical with those of the allied marine form, *Pseudocalanus minutus* Krøyer. *Senecella calanoides* females possess only four legs and sex was therefore determined at the 4th copepodite stage. The nauplii of *S. calanoides* are extremely large and unmistakable although they have not yet been described. Since they were never abundant, and because the younger stages were difficult to separate with confidence, they were grouped into three categories: nauplius 6, showing buds of the first two pairs of legs; nauplii 4–5, showing prominent 1st maxillae; and nauplii 1–3, lacking these appendages of the older stages and being smaller and oval in shape.

Temperatures were determined with an Atkins thermistor and sensing probe, usually at 2-m intervals to 30 m, at 5-m intervals to 50 m, and at 10-m intervals to the bottom. On May 22 and September 23, 1968, temperatures could not be taken because of defective apparatus.

TEMPERATURE

Thermal stratification was fully established by mid-June in both years of the study (Fig. 2). In 1967 the Sound was circulating vertically by December 7 and was again turning over on April 22, 1968, shortly after departure of the ice. Hypolimnetic temperatures reached an observed maximum on November 11, 1968; surface waters had by this time cooled below 10 C. Summer temperatures at all depths below the epilimnion were somewhat higher in 1968 than in 1967: the thermocline was deeper and the hypolimnion warmer earlier in June, July, and August of 1968 than in each of the same months of 1967.

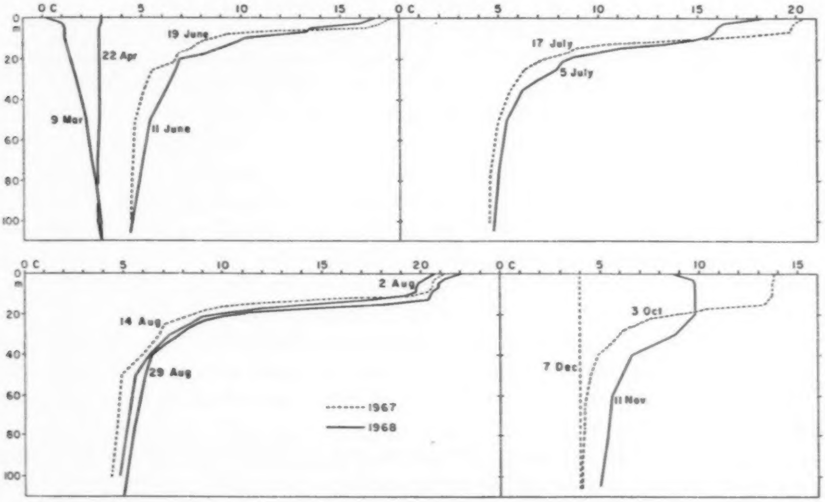

FIG. 2. Vertical distributions of temperature in Parry Sound on 12 occasions from June 19, 1967, through November 11, 1968.

CYCLOPOIDA

Cyclops bicuspidatus thomasi S. A. FORBES

This species was present in the plankton on all dates and was usually the most abundant cyclopoid (Fig. 3). In 1967 two peaks, in June and December, and in 1968 three peaks, in April, August, and November, are noted. The maximum occurred in midsummer only in 1968 and possibly the higher water temperatures in that year, by accelerating development, contributed to the outburst. Andrews (1953) reported *C. b. thomasi* absent from western Lake Erie from early summer to early winter during the 2 years of his study. Davis (1962), however, recorded it throughout the year, with a July maximum, in the Cleveland Harbor area of Lake Erie. Wells (1960) also found it present

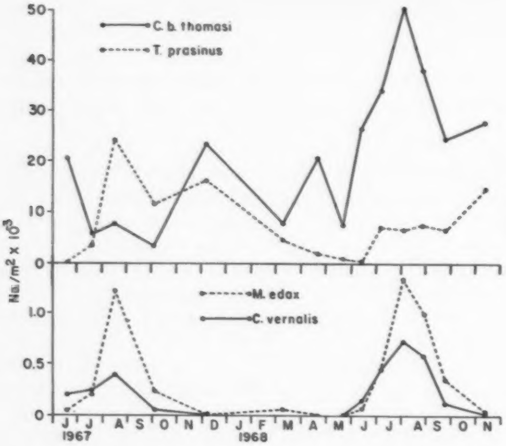

FIG. 3. Seasonal abundance of adults of four cyclopoid copepods in Parry Sound, June 1967–November 1968.

at all times from early June to mid-November in Lake Michigan, the maximum occurring in early summer. Thus, *C. b. thomasi* would appear to be a basically winter and spring species, which can become year-round when conditions are suitable.

Except at times of vernal and autumnal circulation, when *C. b. thomasi* was distributed fairly evenly throughout the water column, greatest numbers were found in the upper strata (Fig. 4). Concentration directly beneath the

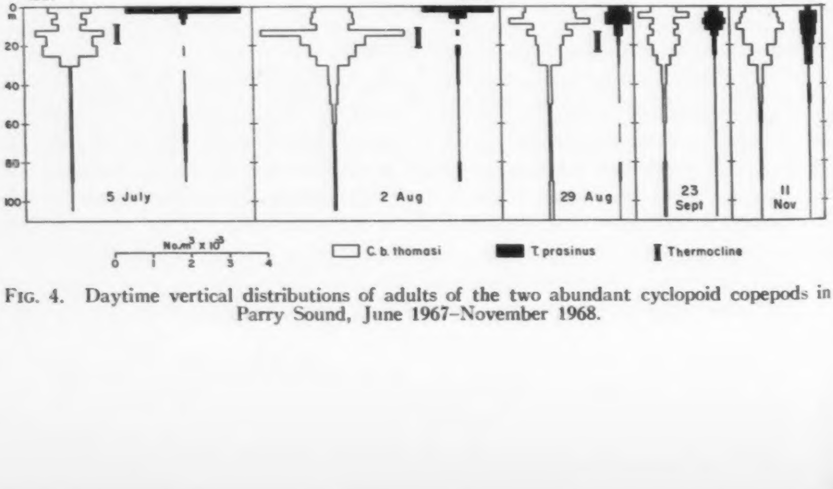

FIG. 4. Daytime vertical distributions of adults of the two abundant cyclopoid copepods in Parry Sound, June 1967–November 1968.

ice on March 9, 1968, is noteworthy and suggests either a reaction to the weak light or, more probably, a food source at this level.

TROPOCYCLOPS PRASINUS (FISCHER)

This species was also present at all times but, except during the summer of 1967, was less abundant than *C. b. thomasi* (Fig. 3). Peaks occurred in midsummer and late autumn, with minima in June of both years. Males were not noted in any samples from December 7, 1967, through June 19, 1968, nor did females carry egg sacs during this period; males were again scarce in November 1968. Because of its small size *T. prasinus* passes through nets with apertures greater than about 0.16 mm and its appearance in the records is consequently irregular, although Marsh (1909) described it as common in all the Great Lakes. Chandler (1940) found it from April to October in western Lake Erie with a maximum in August; Davis (1962) reported it present throughout the year but never common in the Cleveland Harbor area.

Of all copepods in Parry Sound *T. prasinus* had the greatest concentration near the surface, particularly during summer: in July and early August 1968, only a very small percentage were below 3 m (Fig. 4).

CYCLOPS VERNALIS FISCHER

Cyclops vernalis was found only in warmer months and its numbers were never great (Fig. 3). Although it attains irregular abundance in Lake Erie (Andrews, 1953; Davis, 1962, 1968), it has never been reported common in the Upper Lakes. It was not listed by Eddy (1943) for Lake Superior, and Wells (1960) regarded it as scarce in Lake Michigan. I have found *C. vernalis* abundant in some lakes of high total dissolved solids in southern Ontario, as did Whittaker and Fairbanks in southeastern Washington (1958). Its occasional abundance in Lake Erie suggests it may prefer a more eutrophic environment than that of Parry Sound.

MESOCYCLOPS EDAX (S. A. FORBES)

This large cyclopoid also occurred mainly in summer and was only slightly more common than *C. vernalis* (Fig. 3). It is usually the most abundant cyclopoid in summer and early autumn in Lake Erie (Andrews, 1953; Davis, 1962, 1968). Wells (1960, 1968) found it very abundant in Lake Michigan during the summers of 1954 and 1955 but scarce in 1966 and attributed this decline to alewife predation. I have found *M. edax* in extreme abundance in fish-free acid ponds near Georgian Bay and it may be that predation is a factor where it is scarce in the Upper Lakes.

CALANOIDA

DIAPTOMUS ASHLANDI MARSH

This was the most common diaptomid at all times except the final sampling date (Fig. 5). During 1967 two peaks were observed, in July and December.

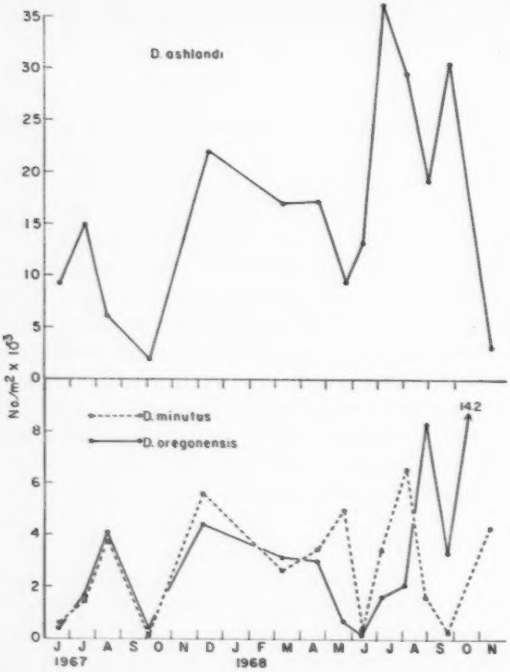

FIG. 5. Seasonal abundance of adults of three species of *Diaptomus* in Parry Sound, June 1967–November 1968.

In 1968 the July maximum again occurred but was succeeded by a second summer pulse in September and then by a steep decline similar to that in the autumn of 1967. *Diaptomus ashlandi* has been reported in all the Great Lakes except Ontario and is usually the most abundant diaptomid in summer in lakes Erie and Michigan (Robertson, 1966). Curiously, Sars (1915) did not mention the species as occurring in Go Home Bay, Georgian Bay.

DIAPTOMUS MINUTUS LILLJEBORG

This species was usually much less abundant than *D. ashlandi* (Fig. 5). In 1967 peaks occurred in August and December and minima in June and October. In 1968 a spring peak was reached in May followed by a June minimum, a summer maximum in early August, and an autumn minimum in late September. Numbers then peaked again in November. *Diaptomus minutus* has been reported in most Great Lakes collections and is often the most abundant diaptomid in spring. I have found that *D. minutus* in the small, shallow lakes bordering Georgian Bay is usually a winter–spring form, being either replaced or exceeded in numbers in summer by *Diaptomus reighardi*.

DIAPTOMUS OREGONENSIS LILLJEBORG

This species had approximately the same abundance as *D. minutus* (Fig. 5). In 1967 a summer maximum occurred in August, after which numbers declined abruptly only to peak again in December. Numbers then fell off slowly during the first half of 1968 to reach a minimum in June. Peaks were then attained in midsummer and late autumn as in the previous year. *Diaptomus oregonensis* has been recorded from all the Great Lakes including Georgian Bay. It is one of the most common species in Lake Erie (Davis, 1968) but its importance appears to decrease in the upper lakes, and in Lake Michigan it is abundant only in autumn (Robertson, 1966).

DIAPTOMUS SICILIS FORBES

Diaptomus sicilis was always scarce, only a few adults being found in the combined samples of any date. Most specimens were taken in the cooler months and none in the summer of 1968. Although fairly abundant in lakes Michigan (Wells, 1960) and Superior (Eddy, 1943), *D. sicilis* may not be common in any part of Georgian Bay since Sars (1915) gives it no mention in his records of Go Home Bay plankton. It is generally accepted as a coldwater species and its scarcity in Parry Sound suggests it may also be mainly an offshore form as Rawson (1956) found in Great Slave Lake.

VERTICAL DISTRIBUTION OF *DIAPTOMUS* SPP.

When two or more species of *Diaptomus* coexist in reasonable numbers there is commonly some degree of vertical separation between the adults. In southern Ontario lakes Rigler and Langford (1967) found that when *D. minutus* and *D. oregonensis* coexisted alone the average depth was usually less for the former than for the latter, and that when a third species was present the adults of it were usually at the greatest depth. In Parry Sound there was clearly little separation between *D. ashlandi* and *D. oregonensis* adults at any time (Fig. 6). Both were mainly metalimnetic during periods of extreme thermal stratification and occupied somewhat deeper strata in spring and fall. Although the distribution of *D. minutus* overlapped those of the other two species considerably this animal usually showed greater concentration in the epilimnion than the others. This was particularly pronounced throughout both summers but occurred also on March 9, 1968, during winter stratification, and on May 22, 1968, when summer stratification was probably still light. During the two periods of turnover, December 7, 1967, and April 22, 1968, all three species were distributed fairly uniformly from surface to bottom with no marked concentration at any depth.

EPISCHURA LACUSTRIS FORBES

This species was never common at any time. A few specimens were present in each series from June through December 1967, and again from July through November 1968. It was absent in samples from winter and spring of 1968 and is known to produce autumn-resting eggs (Main, MS, 1962). *Epischura lacustris* has been reported from all the Great Lakes and described as abundant in Georgian Bay (Sars, 1915), and lakes Superior (Eddy, 1943) and Michigan (Wells, 1960).

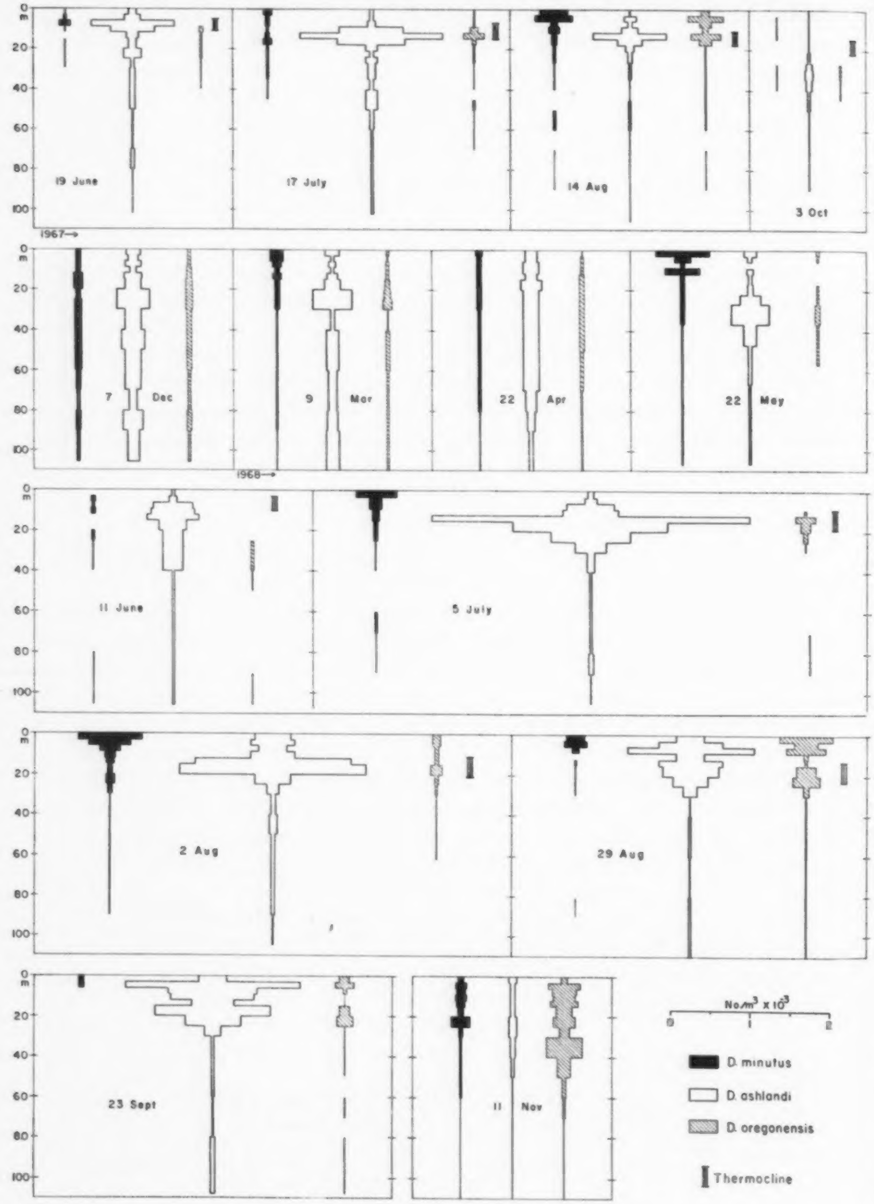

FIG. 6. Daytime vertical distributions of adults of the three abundant species of *Diaptomus* in Parry Sound, June 1967–November 1968.

EURYTEMORA AFFINIS POPPE

Eurytemora affinis was the rarest of all copepods with a maximum of nine adult specimens being taken on August 2, 1968. Although it has been recorded since 1954 for all the Great Lakes except Superior very little is known of its ecology in fresh water. It is at least in part a littoral form since I have found it fairly common in the shallows along the Georgian Bay coast. On the other hand Wells (1968) reported markedly greater numbers in the offshore than in the inshore waters of Lake Michigan.

LIMNOCALANUS MACRURUS SARS

Limnocalanus macrurus has been reported from all the Great Lakes including Georgian Bay. It is usually abundant except in western Lake Erie where summer temperatures are probably too high. In Parry Sound it was the most abundant deepwater copepod at all times.

LIFE CYCLE

Limnocalanus macrurus females do not carry egg sacs; the eggs are extruded directly into the water, sink to the bottom, and hatch after a resting period of varying length (Hutchinson, 1967). The first naupliar instar was very rarely found in Parry Sound and may live close to the bottom where it escapes capture by vertical hauls.

Limnocalanus macrurus in Parry Sound was found to have one generation annually although the actual period of development lasted considerably less than a year. Adults were much more abundant than any other stage except from November to May (Fig. 7 and Table 1). On June 19, 1967, 45% were C6 though numerous nauplii were still present; 2% of females, probably survivors of the previous generation, bore spermatophores. On August 14, 88% were C6 but no females yet bore spermatophores. On October 3 virtually all *L. macrurus* were C6; breeding had commenced since 11% of females bore spermatophores and a few N2 were present. By December 7 the total number of adults had decreased greatly; all naupliar stages were now present, the mode being at N2. By March 9, 1968, the most advanced members of the new generation had reached at least C5 and possibly some were already at C6. Development proceeded rapidly during the next 3 months and, on June 11, 65% had reached C6. The rare females with spermatophores on this date probably belonged to the old generation. By July 5, 84% of the population were C6. This contrasts with 60% on July 17 the previous year and suggests that the slightly higher water temperatures in 1968 (Fig. 2) may have accelerated development. The population structure continued relatively static the remainder of the summer while the last copepodites and nauplii slowly disappeared. On August 29, 2% of females bore spermatophores indicating breeding had commenced. By September 23, 16% bore spermatophores; a few N2 were present suggesting the resting period of the eggs may be fairly brief. On November 11 spawning was much in evidence: 55% of females bore spermatophores and numerous N2 and N3 were present.

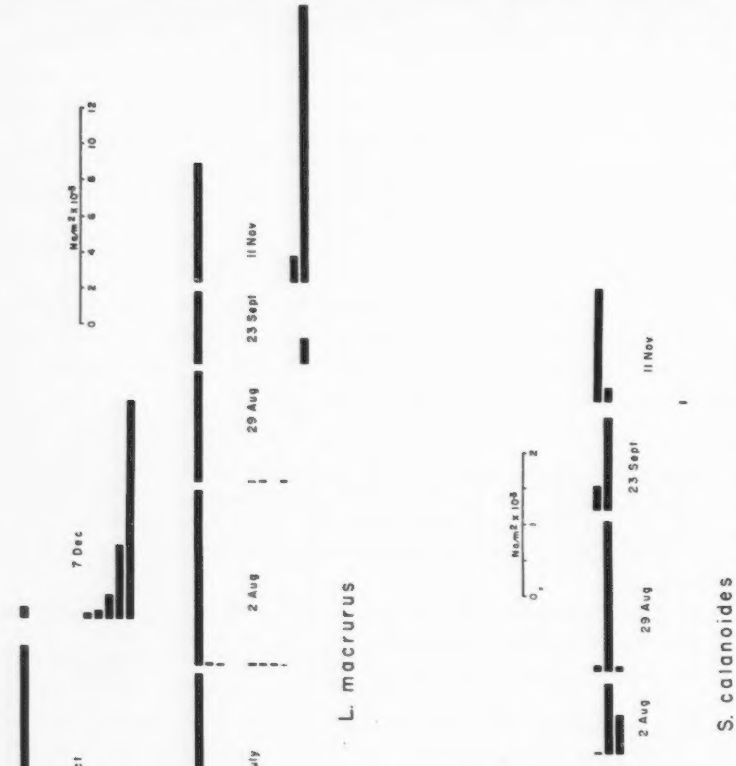

FIG. 7. Seasonal abundance of naupliar and copepodite stages of *Limnocalanus macrurus* and *Senecella calanoides* in Parry Sound, June 1967–November 1968.

SEX RATIOS

On dates when sufficient specimens were available, sex ratios of neither C5 nor C6 (Table 1) deviated significantly from one ($P < .05$) indicating that both moulting rate and numbers hatching were similar for the two sexes. The practically uniform ratios also suggest that the rates of adult mortality were approximately equal for both sexes during the period. These findings accord generally with those of Comita (1956) for *Limnocalanus johanseni* and Lindquist (1961) for *L. grimaldii.*

VERTICAL DISTRIBUTIONS

The daytime vertical distributions of copepodites of *L. macrurus* differed greatly from that of the adults (Fig. 8) though there is little evidence that the distributions differ in other limnetic copepods. Except on April 22, 1968, when the water was circulating vertically, both the modal and the median depths of most copepodite stages tended to be above those of each immediately older stage. Whether this represents an actual preference of the younger stages for the upper depths or merely indicates a physical incapacity to migrate deeper for the day is uncertain. The heavy concentrations of stages C1, C2, and C3 directly beneath the ice on March 9, 1968, would certainly suggest that the animals were drawn there by a preference, possibly for a type of food though the additional influence of light cannot be discounted.

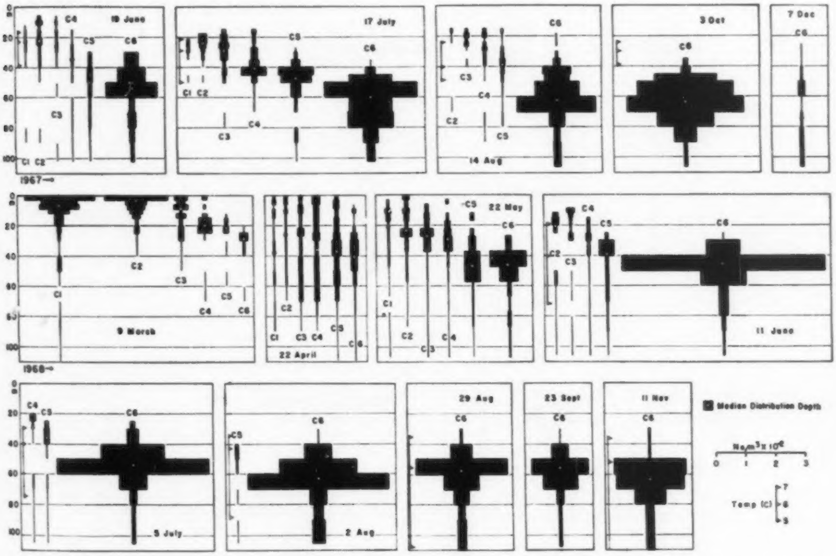

FIG. 8. Daytime vertical distributions of copepodite stages of *Limnocalanus macrurus* in Parry Sound, June 1967–November 1968.

As the water warmed after spring turnover all stages occupied somewhat deeper strata, although copepodites 1–5 were seldom plentiful below 50 m. Daytime vertical distribution of adults was usually confined to middle depths. A modest summer descent of adults occurred both years, modal depth dropping from 50–60 m on June 19, 1967, to 60–70 m on October 3 and from 37–47 m on May 22, 1968, to 60–70 m on November 11. The daytime upper temperature limit of the adults was about 7 C, only a few individuals occasionally occurring above this level.

The possible effect of *S. calanoides* on the vertical distribution of *L. macrurus* deserves consideration. Comparison of Fig. 8 and 9 shows that, although there was often a considerable overlap of the depths occupied by the two species, the modal and median depths of the various stages of *L. macrurus* were almost always distinctly higher than those of the predominant *S. calanoides* stages. On the other hand Martin and Chapman (1965) found that in the region of Algonquin Park, Ont. *L. macrurus* was usually found in deeper lakes than *S. calanoides*.

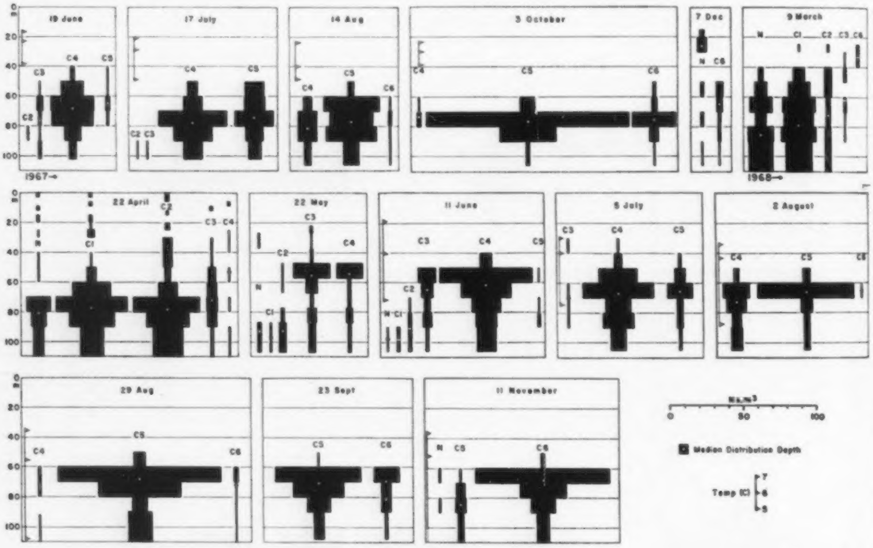

FIG. 9. Daytime vertical distributions of copepodite stages and total nauplii of *Senecella calanoides* in Parry Sound, June 1967–November 1968.

SENECELLA CALANOIDES JUDAY

Described only in 1923, *S. calanoides* is characteristic of many deeper lakes of central and eastern Canada and northeastern USA, but is seldom abundant. It has been recorded in all the Great Lakes except Erie. In Parry

Sound, it was always less numerous than *L. macrurus* with usually about one-fifth the population on any date.

LIFE CYCLE

The eggs of *S. calanoides* have not yet been found but it is probable that, like those of *L. macrurus*, they are extruded directly into the water and sink to the bottom before hatching.

Although monocyclic, the life history of *S. calanoides* in Parry Sound differed considerably from that of *L. macrurus* in three important aspects: firstly, spawning was later by approximately 6 weeks judging by comparative percentages of females carrying spermatophores in the two species (Table 1); secondly, development rates were much slower in *S. calanoides* and consequently it did not reach C6 until 4–5 months after *L. macrurus* (Fig. 7); finally, moulting of individuals within each instar, particularly the older ones, was more nearly synchronous than in *L. macrurus* so that the population on most dates was concentrated in one or two stages only.

On June 19, 1967, when nearly half of *L. macrurus* were at C6, *S. calanoides* was concentrated mainly at C4 (Fig. 7 and Table 1). On August 14, C5 was predominant; a few animals were at C6 though no females yet bore spermatophores. On October 3, 18% had reached C6 but only 3% of females carried spermatophores and no nauplii had yet appeared. During the next 2 months the remaining copepodites presumably matured and spawned since by December 7 the population was reduced to a few adults and younger nauplii. The subsequent 3 months was a period of maximum hatching and development of younger stages as evinced by the large mode at C1 on March 9. By June 11, nauplii 1–5 had disappeared and the mode was now at C4. Adults of the new generation first appeared on August 2 but no females bore spermatophores before September 23. On the final sampling date, November 11, 90% of the population had reached C6, spermatophores were borne by 15% of females, and the first few nauplii had hatched. That higher water temperatures in 1968 had also accelerated development in *S. calanoides* is suggested by the fact that, on September 23, 21% of the population were at C6 contrasted with only 18% on October 3, 1967.

SEX RATIOS

Sex ratios of stages C4, C5, and C6 (Table 1) deviated significantly from one ($P < .05$) on two occasions only (C5, July 17, 1967; and C4, August 2, 1968) and this is insufficient evidence that the moulting rate in *S. calanoides* was consistently higher in either sex of any stage or that the numbers of the two sexes produced were not approximately equal.

VERTICAL DISTRIBUTIONS

Senecella calanoides (Fig. 9) had a daytime distribution that differed markedly from that of *L. macrurus* (Fig. 8). With very few exceptions the median depths of all stages were below 60 m. Even at times of vertical circulation, December 7, 1967, and April 22, 1968, the great majority of animals were at depths suggesting active maintenance of position through downward swimming. Even the nauplii, plotted as a single group in Fig. 9, were concentrated in deep water except on December 7. Also, in contrast to *L. macrurus*, there was no tendency for copepodites to descend to greater depths with age. Indeed the opposite was more often the case with median depths of younger instars usually greater than those of the older ones.

DISCUSSION

Both *L. macrurus* and *S. calanoides* are monocyclic in Parry Sound. *Limnocalanus macrurus* develops rapidly and reaches C6 in the spring but does not become sexually mature until late summer. In Ekoln, Sweden, Ekman (1907) reported a similar delay in reaching sexual maturity. In Char Lake, Cornwallis Island (75°N, 95°W), however, studies presently being undertaken reveal that development is much slower and that *L. macrurus* does not reach C6 until midsummer (F. H. Rigler, personal communication). As development rate of copepods is a positive function of temperature (McLaren, 1963), undoubtedly the faster rate of *L. macrurus* in Parry Sound than in Char Lake is due to higher water temperatures, especially since it has been shown that younger instars are often distributed in the warmer upper waters. In addition, adult *L. macrurus* are known vertical migrants (Marsh, 1897; Wells, 1960) and it may be that pre-adult instars also move toward the warmer surface waters at night. However, despite its rapid development in Parry Sound, *L. macrurus* is unable to attain early sexual maturity to produce a second annual generation and so must live through the summer without breeding. Because of this lengthy period of C6 immaturity, with the attendant dangers from predation, parasitism, disease, and starvation, *L. macrurus* in Parry Sound would seem maladapted. Yet C6 mortality was low, even allowing for major sampling errors, since a population of 6–10 $\times 10^3/m^2$ survived relatively intact through both summers. However, Norden (1968) has shown that in Lake Michigan, *L. macrurus* adults are in fact preyed upon extensively by the larvae of the alewife, *Alosa pseudoharengus*.

The life history and patterns of vertical distribution of *S. calanoides* differed considerably from those of *L. macrurus*. It became adult in the autumn after a lengthy development period and spawned almost immediately. There was little observable tendency for any instars to inhabit the upper strata during the day, and evidence from Lake Michigan (Wells, 1960) indicates the impulse to migrate into surface waters at night may be weak. By reason of its longer period of development, *S. calanoides* seems much better adapted than *L. macrurus* to life in Parry Sound.

Limnocalanus macrurus is an arctic relict, having been originally sluiced up from northern seas and transported south as part of the fauna of proglacial lakes (Segerstråle, 1962). It is characteristic of many deeper lakes of Eurasia and also of some lakes of the Canadian arctic islands. *Senecella calanoides* is also considered a glacial relict, but its earliest origins in North America are unknown. Present southern limits of distribution in North America are similar to those of *L. macrurus*, but it has not been recorded north of Great Slave Lake and it may find true arctic conditions unfavourable. Possibly it does not occur in the high arctic because the extremely low water temperatures throughout most of the year would extend its life cycle beyond 12 months and because it is unable to make the major physiological adjustment to a biennial cycle. On the other hand *L. macrurus* may be essentially arctic adapted and less suited for life in temperate lakes generally than *S. calanoides*. It is suggestive that of the 63 lakes in the region of Algonquin Park, Ont., examined by Martin and Chapman (1965) 45 contained *S. calanoides* and only 22 *L. macrurus*; also, of 11 Finger Lakes of New York State *S. calanoides* has been found in 4 and *L. macrurus* in only 1 (Hall and Waterman, 1967).

ACKNOWLEDGMENTS

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Body Size in White Whales, *Delphinapterus leucas*

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Measurements of length, girth, and weight show that male white whales grow larger than females. The smallest white whales come from western Hudson Bay, the White Sea, and Bristol Bay, Alaska. Animals of intermediate size inhabit all other arctic Canadian localities sampled and also the St. Lawrence River and the Kara and Barents seas. The largest white whales inhabit West Greenland waters, the Okhotsk Sea, and the coast of Sakhalin. Extreme differences in body weight of adult males are about threefold. Nonoverlapping differences in size indicate isolation of some adjacent populations of white whales; equal or overlapping sizes suggest, but cannot prove, mixing of other populations. Size can be positively correlated with marine productivity, being lowest in the arctic and in estuaries and highest in subarctic seas. Since white whales most often grow largest at the southern ends of their range, their restriction to the arctic is attributed either to competition with certain of the Delphinidae or to predation from killer whales, *Orcinus orca* L., or to both. Both putative competitors and predator lack adaptations for arctic life.

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INTRODUCTION

THE ORIGINAL PURPOSE of this study was to examine variation in body size in white whales, *Delphinapterus leucas* Pallas. White whales and narwhals, *Monodon monoceros* L., are the only living members of the Monodontidae, a family of primitive or ancient whales now inhabiting only arctic seas. The results led to interesting conclusions on the probable segregation of different populations, the relationship between body size and environmental productivity, and the factors that adapt white whales to life in arctic waters.

Because of the various sampling errors, accurate criteria of length had first to be found.

MATERIALS AND METHODS

Between 1955 and 1968, the authors and other members of the Arctic Biological Station (formerly Arctic Unit) of the Fisheries Research Board of Canada, together with other Canadian biologists, examined and measured several hundred white whales from various localities in the Canadian Arctic.

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In addition to these previously unpublished measurements, all length measurements or length frequencies of white whales hitherto published and known to us have been included in this study, in order to obtain circumpolar coverage.

Localities of material (Table 1, Fig. 1) include the major part of the range of white whales where fisheries take place, with the exception of Spitsbergen for which we can find no length data.

TABLE 1. Sources of measurements of white whales used in this study.

Locality	Lat	Long	Years of study	Netted (N) or shot (S)	Source of data
1. Churchill, Hudson Bay	58°40′N	93°10′W	1955–56	S	Original
2. Whale Cove, Hudson Bay	62 10	92 30	1962–64	N	"
3. Clearwater Fiord, Cumberland Sound, Baffin Is.	66	68	1966–67	N	"
4. St. Lawrence R.	49	68	1938–39	S	Vladykov, MS, 1944
5. Ellesmere, Cornwallis, and Somerset Is.	76 25	84	1962–67	S	Original
6. Sukkertoppen, West Greenland	65 25	53	1926–28	N	Degerbøl and Nielsen, 1930
7. White Sea	66 30	33	1934	N	Provorov, 1967
8. Kara and Barents seas	73	80	1955–57	?	Kleinenberg and Yablokov, 1960; Khuzin, 1963; Yablokov, personal communication
9. Okhotsk Sea	55	140	1958	N	Kleinenberg et al., 1964
10. Sakhalin	53	144	1930	N	Dorofeev and Klumov, 1935
11. Bristol Bay, Alaska	59	157	1954–66	S	Brooks, MS, 1954; Lensink, MS, 1961
12. Mackenzie Delta, Beaufort Sea	69	133	1955–68	S	Original

These studies give individual measurements of body length or measurements grouped in length classes of 10 or 20 cm. The resulting length frequencies in 20-cm classes are shown for each locality in Fig. 2–5.

In our own studies, lengths were measured in a straight line from tip of snout to notch of flukes. We cannot be sure that all investigators did the same, and some increase will occur if measurements were made round the curve of the body. However, the independent sets of measurements made by Dorofeev and Klumov (1935) at Sakhalin, and by Kleinenberg et al. (1964) in the Okhotsk Sea come from closely adjacent localities and show excellent agreement. We, therefore, consider that the very large sizes shown by them, and by Degerbøl and Nielsen (1930) are real, and that marked variations in body length, and even more marked variations in body weight, are shown by white whales from different areas.

Samples came from two sorts of fisheries: in about half the samples, animals had been harpooned or shot; in the other half, they had been netted. When shooting or harpooning white

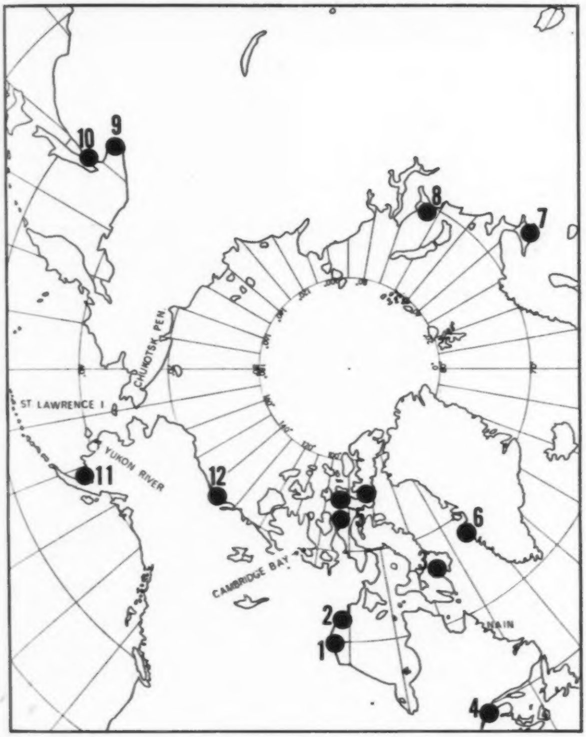
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FIG. 1. Locations of samples of white whales. See Table 1 for key.

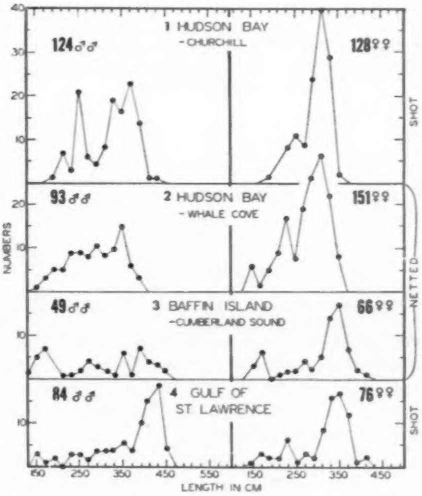

FIG. 2. Length frequencies of white whales from eastern Canada, by 20-cm length classes. Data for the Gulf of St. Lawrence from Vladykov (MS, 1944).

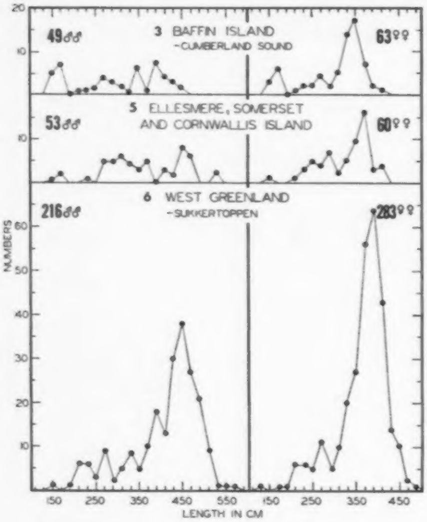

FIG. 3. Length frequencies of white whales from Davis Strait and Baffin Bay. Data for West Greenland from Degerbøl and Nielsen (1930).

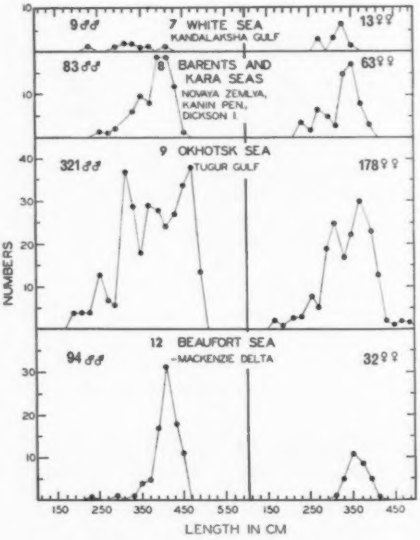

FIG. 4. Length frequencies of white whales from the Soviet arctic. Data for the White Sea from Provorov (1957); for the Kara and Barents seas from Kleinenberg and Yablokov (1960) and A. V. Yablokov (personal communication); for the Okhotsk Sea from Kleinenberg et al. (1964).

whales, hunters tend to select the larger animals, and since male white whales are larger than females, this results often in a high percentage of males in the sample. Net fisheries, on the other hand, catch all sizes of animals. If selection occurs it is usually towards the smaller animals because nets are usually set close to shore where they capture females with calves and miss the larger animals that avoid shallow water.

These differences are exemplified in our two samples from western Hudson Bay, which were taken from localities 300 km apart; that from Churchill (locality 1) by shooting, and that from

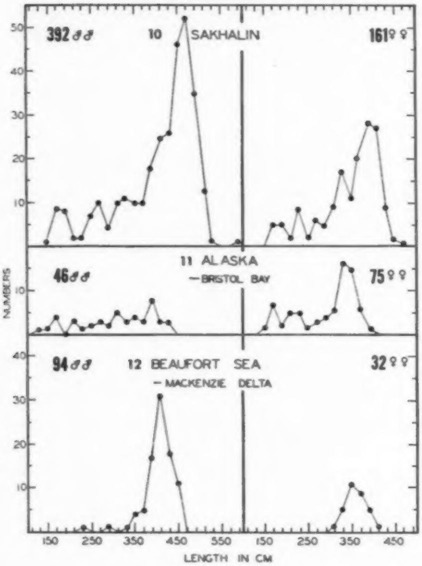

FIG. 5. Length frequencies of white whales from the North Pacific and the Pacific sector of the Arctic Ocean. Data for Sakhalin from Dorofeev and Klumov (1935); for Bristol Bay, Alaska from Lensink (MS, 1961) and Brooks (MS, 1954).

Whale Cove (locality 2) by netting. Recent marking by us has shown that animals move from one locality to the other and that these two populations are therefore either mixed or identical. Nevertheless the sample, taken by net at Whale Cove, shows a lower mean length for both sexes than the sample from Churchill (Fig. 2). (*P* for males and females was < 0.001 in a *t*-test.)

Because of the two kinds of selection, we have separated mean lengths of shot and of netted animals. Means, ranges, and standard deviations are shown in Fig. 6 and 7.

A fairly good comparison between samples can be made using the peak or modal frequency of adults shown in the length-frequency polygons of Fig. 2–5. These peaks represent the accumulation of animals that have ceased body growth, the peaks being more pronounced for females, which cease growing earlier in life than males. The peaks cannot, however, be expressed accurately unless the sample is large and the class interval can be reduced. In practice, a 20-cm interval

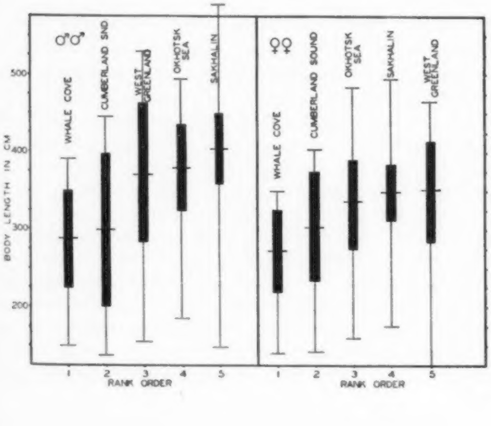

FIG. 6. Mean lengths, ranges, and standard deviations of white whales taken in nets, arranged in order of increasing length.

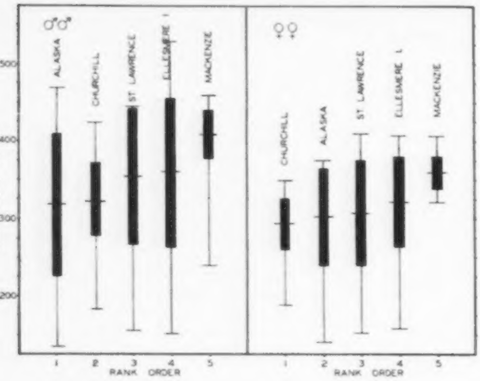

FIG. 7. Mean lengths, ranges, and standard deviations of white whales taken by shooting or harpooning, arranged in order of increasing length.

is necessary to include small samples. Using this method, peak frequencies for the two samples for Hudson Bay (Table 2) show identical values for females and a 20-cm difference between males, with Whale Cove lower than Churchill. Thus the male peak is still affected by selectivity, but the female peak is affected less, since the females are smaller than the males. In Cumberland Sound, Brodie netted his own animals and deliberately caught females for study by setting nets in shallow water. As a result the male peak is almost absent (Fig. 3).

TABLE 2. Modal maximal lengths of white whales in ascending order of size. Data from Fig. 2–5.

Locality	Length (*cm*) Males	Females
Whale Cove, Hudson Bay	350	310
Churchill, Hudson Bay	370	310
White Sea	ca. 400	330
Bristol Bay, Alaska	390	340
Kara and Barents seas	400	340
Cumberland Sound	(–)	350
Mackenzie Delta	410	350
St. Lawrence R.	430	350
Ellesmere Is.	450	370
West Greenland	450	390
Okhotsk Sea	470	370
Sakhalin	470	390

White whales begin life a dark grey in colour but slowly lighten, becoming white at or near to attainment of sexual maturity, but rather later in females than in males. Taking the measurements of only white animals allows us to determine the mean of an approximately symmetrical length distribution with its mode at or very close to the peak in the length-frequency polygon. The ascending limb is the result of variability in age of whitening; the descending limb, of variability in age at death. This mean has the advantage over the mode that it can be expressed with greater accuracy and its variability measured. Colour information was available for all published samples except those for West Greenland. It appears that attainment of the white colour is estimated with comparable accuracy by all observers since most express a grade of colour "white with grey tinge" or "white with blue tinge" as the point of change to white. Means, ranges, and standard deviations for white animals of both sexes are shown in Fig. 8. Means for samples 1 and 2 from Hudson Bay are closely similar for males and fairly similar for females, in spite of the difference in selectivity (for ♂♂, $P > 0.5$; for ♀♀; $0.1 > P > 0.05$) This statistic is therefore little affected by selectivity in sampling, and has the advantage over the total sample mean of a lowered range and standard deviation.

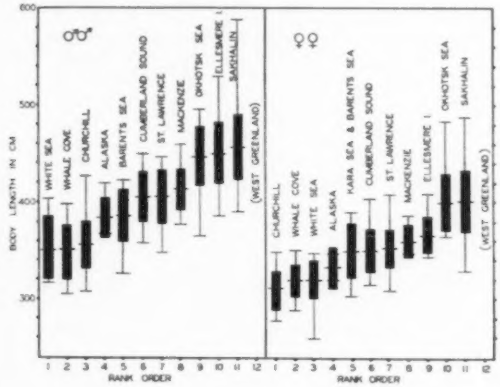

FIG. 8. Mean lengths, ranges, and standard deviations of adult white whales, arranged in order of increasing length.

Adult male white whales often isolate themselves into separate herds (Tarasevich, 1957). An example of the divergence in mean length that may occur is given by Degerbøl and Nielsen (1930) who measured two sets of netted samples of white whales from West Greenland in the same winter. Samples means differed by 31 cm or 7% for males but by only 1 cm or 0.3% for females (P for ♂♂ < 0.001, for ♀♀ > 0.5). The presence or absence of such male herds will greatly affect mean lengths of males but will not greatly affect the peaks in length-frequency polygons of males, nor the mean lengths of white males.

Restriction of measurements to white animals much reduced the size of samples, and this was particularly true for females, which whiten at a later age than males. For all these reasons we conclude that modal maximal length is normally the best available statistic for female white whales, and mean length of white adults the best statistic for male white whales. Where neither statistic is available, mean length of all animals in a sample is still useful.

Growth of two populations may also be compared by means of age–length curves (Fig. 9). Such information was available for most of our own samples and also for a number of female white whales from the Kara and Barents seas studied by Khuzin (1963). Since Khuzin did not give information on colour of Kara and Barents seas females, we have taken information from our own data that female white whales whiten on average at attainment of 22 dentinal tooth layers. Applying this finding to Khuzin's data, and using lengths of females with at least 22 dentinal growth layers, we have calculated the mean length of white females from the Kara and Barents seas (Fig. 8).[1]

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[1] Since we wrote the above, A. V. Yablokov has sent us length and colour data for both sexes of white whales from the Barents Sea. The mean length of white females obtained from the new sample agrees so closely with the mean calculated from Khuzin's data that we have left intact the parameters for females, and added the parameters for males.

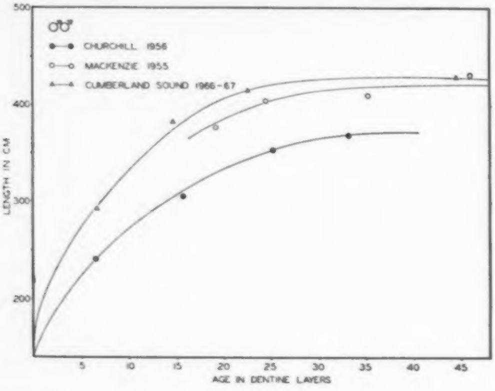


FIG. 9. Growth of male white whales from Churchill, Mackenzie Delta, and Cumberland Sound. Smooth curves are fitted by eye to the least square means for each decade of age (in layers).

Differences in length result in considerably greater differences in weight of individual white whales from different areas, because of the approximately cubic relation of weight to length. We have calculated regression of weight on length for white whales from Churchill and from the St. Lawrence River using published data, and have estimated weights of animals of extreme sizes from these curves. Differences in blubber thickness, also expressed by girths, result in regressions of weight on length having different slopes.

We have information on maximal girth, or circumference, of animals from Cumberland Sound and Churchill. Maximal girth is measured just behind the forelimbs.

Blubber thickness was measured in animals from the same two areas, midlaterally at the neck or above the flippers.

RESULTS

LENGTHS

When females are compared using all three length criteria, white whales from western Hudson Bay (Churchill and Whale Cove) are the smallest of all white whales studied (Fig. 6–9). Close to them come white whales from the White Sea, and slightly larger again, white whales from Bristol Bay, Alaska (Fig. 4–8). White whales of medium size come from all other Canadian arctic localities — the Beaufort Sea, Cumberland Sound, and the region of Ellesmere Island, as well as from the St. Lawrence River — and from the Kara and Barents seas (Fig. 2–9). White whales from Ellesmere Island may be a little larger than most of this intermediate-sized group but larger samples are needed from this region. Largest of all white whales are those from West Greenland and from the region of Sakhalin and the Okhotsk Sea (Fig. 4–8).

In the western North Atlantic sector small white whales occur in western Hudson Bay; intermediate sized animals occur at all Canadian localities and in the St. Lawrence River; and large animals occur on the coast of West Greenland. In the eastern North Atlantic, animals from the White Sea are small and animals from the Kara and Barents seas somewhat larger, although available published samples from each area are small. In the North Pacific, animals from Bristol Bay, Alaska, are small, and animals from the Beaufort Sea are of intermediate size. Animals from the Okhotsk Sea and Sakhalin are very large, equalling those of West Greenland in sizes attained (Fig. 4 and 5).

GIRTHS

A regression of girth on length (Fig. 10) shows clearly that animals of both sexes from Cumberland Sound, starting from a similar body form, become thicker-bodied than animals from Churchill. We cannot distinguish from these data if this results from a lower rate of deposition of blubber in the whales from Churchill, or a smaller formation of muscle, or both, though a lower rate of deposition of blubber seems more probable. We do not have enough weighings from Cumberland Sound to test this. Weighings of parts of a single animal from Ellesmere Island show the same percent of blubber (40%) as in animals from Churchill. But this animal was caught in winter after having been kept close to shore by ice, and its blubber thickness could have declined with starvation.

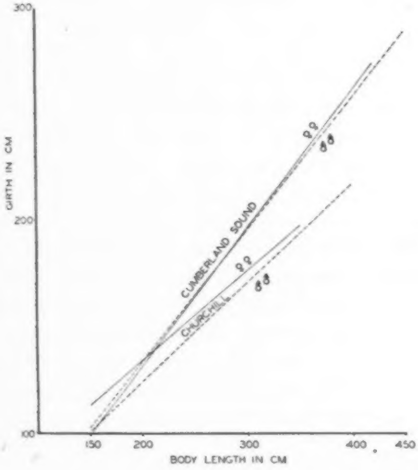


FIG. 10. Regression of girth on body length for male and female white whales from Churchill, Hudson Bay, and Cumberland Sound, Baffin Island.

A separate study of blubber thickness suggests that it is mainly reduced blubber thickness that results in the thinness of body of white whales from western Hudson Bay.

BLUBBER THICKNESS

Blubber thickness was measured and compared with body length (Fig. 11) for male and female white whales from two areas: Churchill in Hudson Bay, and Cumberland Sound.

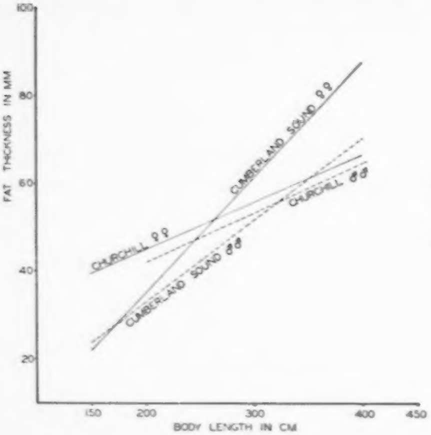


FIG. 11. Increase of blubber thickness with body lengths for male and female white whales from (*a*) Churchill, Hudson Bay, and (*b*) Cumberland Sound, Baffin Island.

The curves for male and female white whales from Churchill show about the same slope, but males are slightly leaner. As length increases, males from Cumberland Sound become fatter than males from Churchill, the rate of increase of blubber thickness with length being greater; the difference is even greater when females from Cumberland Sound are compared with females from Churchill. This result indicates that, as well as attaining a smaller mean size, white whales from Hudson Bay do not achieve the same fatness as animals from Cumberland Sound.

In females taken from Cumberland Sound there is a wide variation in blubber thickness owing to varying reproductive state, females in late pregnancy and early lactation being unusually fat (Fig. 12). The same divergence is seen, probably for the same reason, in an analysis of fatness of white whales from Whale Cove, Hudson Bay, not shown here. Both these samples were from

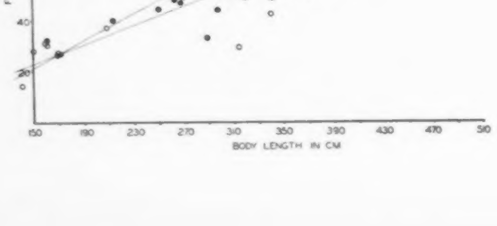


FIG. 12. Blubber thickness plotted against body length for male and female white whales from Cumberland Sound. Regression lines are shown for both sexes.

nets that caught females accompanied by calves. The catch at Churchill, taken by harpoon, included few females in late pregnancy and few young calves, and here, by contrast, blubber thickness was very similar in males and females. In general it seems desirable to compare the rate of increase of blubber thickness in males, in which variation in blubber thickness with reproductive state is small, and which provide a wider range of lengths than females.

WEIGHTS

Because of their lower rate of increase of blubber thickness, white whales from Hudson Bay show a smaller coefficient of regression of weight on length than, for example, animals from Cumberland Sound. Thus it is desirable not to group weight–length data for animals from different localities.

We do not have weights of large animals from Cumberland Sound, but Vladykov (MS, 1944) has published a series of weights and lengths for 10 animals from the St. Lawrence River, which are probably comparable, since the animals from these two populations reach the same absolute sizes (Fig. 3, 8). For Hudson Bay, Doan and Douglas (1953) published lengths and weights for 16 gutted animals. We assume that "gutted" refers to abdominal viscera only, and does not include heart, lungs, and liver. From our own weighings of body parts (Table 3) we estimate that about 5% of the total weight was missing from these gutted animals. We have therefore divided the gutted weights by 0.95 to give total weights for the 16 Hudson Bay animals.

TABLE 3. Relative weights of parts of the body in white whales (mean of three animals from Whale Cove, Hudson Bay, 1962, lengths 237–304 cm), and one pilot whale *Globicephala melaena* (Traill), length 236 cm, from Sergeant (1962, p. 23)

	% of total body wt	
Organ	*Delphinapterus leucas*	*Globicephala melaena*
Blubber and flippers	43.4	29.1
Meat	15.9	34.0
Vertebrae, ribs, and tail	18.7	10.3
Head	9.9	14.7
Liver	2.7	2.6
Heart	0.7	0.4
Stomach and intestines	4.0	5.2
Lungs and larynx	4.0	3.1
Kidneys	0.7	0.6
Total	100.0	100.0

Regression lines of weight on length for the two sets of data are shown in Fig. 13. The calculated formulae are, for Hudson Bay animals:

$$\log \text{wt (kg)} = 2.536 \times \log \text{length (cm)} - 3.345$$

and for St. Lawrence River animals:

$$\log \text{wt (kg)} = 2.605 \times \log \text{length (cm)} - 3.807.$$

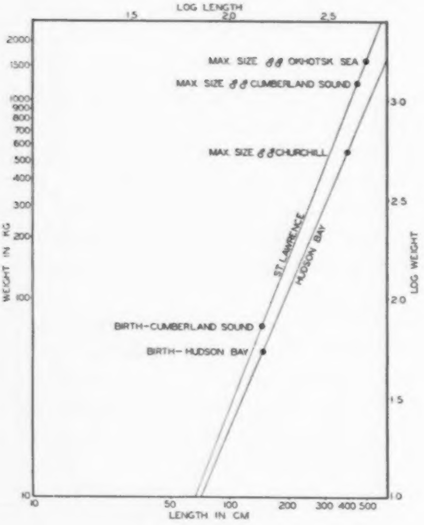


FIG. 13. Regression of weight on length for white whales from Churchill, Hudson Bay, and St. Lawrence River. See text.

On this basis a large male of length 400 cm from Hudson Bay will weigh about 500 kg whereas a large male of length 445 cm from the St. Lawrence River will weigh about 1200 kg, more than twice as heavy. We do not have a series of weighings from a population of large-sized animals, for instance from West Greenland or the Okhotsk Sea, but assuming that they show no further increase in relative fatness we can extrapolate from the curve for animals from the St. Lawrence River. Then a large male of 500 cm from West Greenland will weigh about 1600 kg or nearly three times as much as a large male from Hudson Bay. Thus the ratios of individual biomass among small, medium, and large-sized adult male white whales will be approximately 1:2:3.

DISCUSSION

Klumov (1937) was the first to show that populations of white whales from different areas show markedly different sizes. His table 1 gives mean sizes for white animals, with sexes unseparated, as follows:

	No. of animals	Mean length (*cm*)
White Sea	190	312.6
Kara Sea	300	396
Okhotsk Sea	310	445

On the basis of the very different sizes attained, as well as on some supposed morphological and ecological differences, Klumov separated the three forms as distinct species. This distinction has not been upheld by later Soviet writers. It can hardly be supposed that white whales from the White Sea are fully isolated from animals inhabiting the Kara and Barents seas since Klumov (1939) himself showed a small overlap in range. Nevertheless Khuzin (1960), as a result of winter flights, showed that white whales do winter in the White Sea, suggesting that the stock is resident. Provorov (1957) agreed with Klumov that white whales in the White Sea are smaller than those from the Kara Sea, but did not think the size differences so great.

In the northwest Atlantic also we have distinguished three different sizes of white whales: small animals inhabiting at least the western part of Hudson Bay; medium-sized animals found on all other arctic coasts of Canada, as well as in the St. Lawrence River; and large animals found on the coast of West Greenland.

In summary we may say that small white whales are found in subarctic estuarine conditions, medium-sized white whales in arctic water having some oceanic influence, and large white whales at the southern-most end of the range in subarctic conditions under oceanic influence. Thus on the eastern side of Davis Strait, large-sized white whales in oceanic subarctic water are separated by only 600 km from medium-sized white whales in oceanic arctic water. Because of their different sizes one may assume that these two populations are distinct, possibly with some mixing through the area of Ellesmere Island where animals of a possibly transitional size occur (Fig. 1). The pattern of cold ocean currents would indeed favour diffusion anticlockwise round Baffin Bay by a coastal-living marine mammal, rather than direct crossing of the Bay.

For similar reasons, we may assume that the white whales of western Hudson Bay form an isolated population, not mixing through Hudson Strait, since much greater variation in adult size would otherwise occur. On the other hand similarity in size of all other Canadian arctic white whales, together with those from the St. Lawrence River, gives no grounds for considering one population isolated from another.

In the western Canadian arctic, white whales occur commonly as far east as northern Banks Island, and in the eastern Canadian arctic as far west as Prince of Wales Island. The remaining gap in Viscount Melville Sound is no more than 400 km and could probably be bridged by a few animals in summer when sufficient open water is present. The more southerly route across arctic Canada passes through the gulfs of Amundsen, Coronation, and Queen Maud. Members of the Arctic Biological Station have worked annually from 1964 to 1968 at Cambridge Bay, a central point in this passage. They have recorded one white whale, shot by the inhabitants during this period, as well as one previous but recent record.

The similarity of size between white whales from Cumberland Sound and the St. Lawrence River equally gives no evidence for total isolation of the latter population, although we have no intermediate strandings to indicate supply southward with the Labrador Current. However, white whales occur regularly in Ungava Bay, and we also have verbal reports from Nain, Labrador, of white whales wintering in March between the fast ice and sea ice. This observation serves considerably to reduce the gap between the ranges.

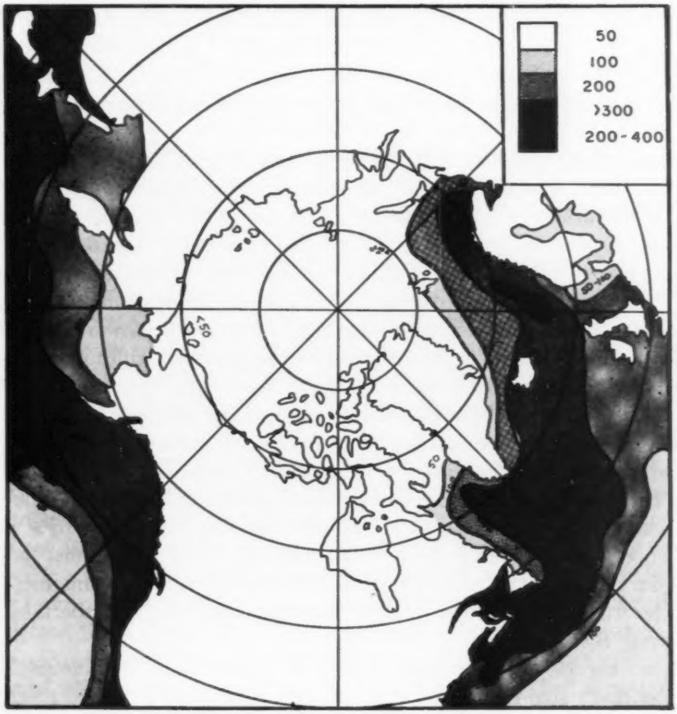
An exception to these generalisations is provided by the white whales from Bristol Bay, Alaska. Although at the southern end of the species' range in Alaska and with a rich diet, which, according to Brooks (MS, 1954), includes much red salmon *Oncorhynchus nerka* (Walbaum)) and other species of Pacific salmon, the size of adult animals is small. Only animals from Hudson Bay and the White Sea are smaller in mean size. It is difficult to account for this circumstance except to assume, firstly, that considerable genetic mixing may occur with arctic animals through the Bering Sea, which provides no physical barriers to such mixing and, secondly, that the winter habitat is restricted by geography.

Lensink (MS, 1961) quotes F. H. Fay (personal communication) to the effect that wintering white whales were seen round St. Lawrence Island in November–December 1967, 2 weeks ahead of formation of pack ice, and Nikulin (1961) notes wintering of white whales of arctic origin in November–December round the Bering Sea coasts of Chukotsk Peninsula. There are also white whales in the estuaries of the Yukon and Kuskokwim rivers, presumably resident.

White whales from Bristol Bay presumably do not pass beyond the Alaska Peninsula and thus live in winter in an estuarine environment. In contrast Nikulin quotes V. A. Arsen'ev to the effect that the white whales of the Amur Region probably winter in the southern part of the Okhotsk Sea and the nearby waters of the Pacific Ocean. Possibly the productivity of the winter environment is the most important factor determining the size attained by white whales. If the winter environment is estuarine, as in western Hudson Bay, the White Sea, and Bristol Bay, the animals remain small.

We suppose that selection has reduced the biomass of an individual white whale to that enabling it to maintain its metabolic activity on the available food. In a suboptimal environment, a larger individual cannot maintain life, so that selection will be for small size. It is necessary to show that Hudson Bay, for example, is a suboptimal environment for white whales.

The accompanying Fig. 14, modified from Hela and Laevastu (1961), shows estimated organic production over the northern hemisphere. Productivity is seen to decrease steadily from north temperate to arctic seas. Although arctic productivity is well known to be low, there are few figures showing variation of production within the Arctic. In Hudson Bay, Lubinsky (1958) showed that the mussel *Mytilus edulis* L. grows to a smaller size at Churchill than in Hudson Strait or in Ungava Bay. Factors cited by Lubinsky as depressing the growth rate of *Mytilus* include the short growing season of the Arctic in general, the low salinity of Hudson Bay, and the absence of mixing between the waters of the Bay and oceanic water. Presumably in brackish water the



ESTIMATED ORGANIC PRODUCTION IN gC/M²/YEAR
(FROM HELA AND LAEVASTU 1961)

FIG. 14. Estimated organic production for northern seas (grams/meter^2 per year), modified from Hela and Laevastu (1961).

plankton available to filter feeders is reduced. Squires (1967) studied the decapod Crustacea of Hudson Bay, including many species widespread in subarctic seas. He showed that many constituent species were smaller or matured later in Hudson Bay than the same species in adjacent Ungava Bay, which is low arctic and estuarine but on the edge of oceanic water, or even than those in Foxe Basin, which has a high arctic but more oceanic climate. This example is interesting since these decapod Crustacea form an important part of the food of white whales in late summer at Whale Cove, Hudson Bay, and a number of Squires' specimens were taken from stomachs of white whales. Thus, populations of small white whales feed on small food items in a suboptimal environment.

Zenkevich (1963) states that the White Sea has an impoverished fauna owing to its low salinity and harsh winters and is considerably less productive than the Barents Sea into which it opens. Klumov (1939) showed that white whales from the Kara Sea winter in the Barents Sea, whereas we have seen that white whales in the White Sea are probably resident. The difference in winter environment could probably account for the difference in mean adult size.

An interesting parallel with white whales is provided by the studies of Jonsgaard (1952) who showed that fin whales, *Balaenoptera physalus* L., from the North Atlantic and North Pacific are equal in size attained, as shown by comparison of animals of each sex that have attained physical maturity, i.e., ceased to grow in length. However, fin whales from the Antarctic attain considerably larger sizes. These differences in size again bespeak a difference in the nutritive conditions of the environment and indicate isolation of populations.

There appear to be no gross differences in numbers of white whales between trophically suboptimal and more suitable environments; the difference is expressed in individual biomass. Thus, in western Hudson Bay, a summer concentration of white whales occurs between the Nelson River estuary and the boundary of Manitoba and the Northwest Territories. This concentration has been estimated by Sergeant to number about 10,000 animals. One obtains the impression from aerial surveys and the distribution of fisheries that the abundance of white whales along this estuarine coast in summer is just as great as in certain high arctic marine environments, e.g., Somerset Island, and greater than in some low arctic environments, e.g., Cumberland Sound and Ungava Bay.

Rosenzweig (1968) studying terrestrial mammalian carnivores has also shown that body size is positively correlated with total primary productivity so that meagre food supplies and not competition set upper limits to body size. However, he did not find prey size to be highly correlated with carnivore body size.

CONCLUSION

White whales grow best at the southern extremity of their range and in more oceanic environments, whereas their typical habitat is estuarine and arctic.

This conclusion suggests that white whales are restricted to their suboptimal environment by biotic factors. These could be either competition or predation. Possible competitors would be species of the later-evolved and widely radiated modern Delphinidae. The latter cannot penetrate to the Arctic because they do not possess the necessary adaptations. Arctic adaptions of the white whale include a thick dermis (Bonin and Vladykov, 1940) and absence of a dorsal fin, both allowing the animals to break new-formed ice with their backs, and a very thick blubber layer giving thermal insulation.

Table 3 shows weights of parts of the body in three white whales from western Hudson Bay compared with a single pilot whale *Globicephala melaena* (Traill) from the boreal waters of Newfoundland (Sergeant, 1962). Males of the pilot whale attain a length of 610 cm, somewhat larger than the largest male white whales. White whales have over 40% of body weight as blubber and pilot whales 30%. As a result, the amount of muscle is much reduced in white whales, 16% compared with 34% in pilot whales. The amount of muscle is less in absolute as well as relative terms. Thus a white whale of length 237 cm weighing 288 kg had 130 kg of blubber and 42 kg of muscle, whereas a pilot whale of length 236 cm and weight 203 kg had 55 kg of blubber and 70 kg of muscle. Nevertheless the weight of skeletal material including the head is larger in a white whale than in a pilot whale.

The blubber layer is also 40% of body weight in *Phocoena phocoena* (Slijper, 1958). This porpoise ranges as far north as its metabolic rate allows (Kanwisher and Sundnes, 1965). The reduction of muscle in white whales and harbour porpoises may mean that the species have lower muscular power, and hence swimming speed or diving ability, than typical Delphinidae. This could put them at a disadvantage in any environments but those to which competitors cannot penetrate.

The largest white whales are not, as one might expect, the most northerly but usually the most southerly, in terms of marine climate. Yet, small animals from Hudson Bay are exposed to as cold a winter temperature, at the freezing point of sea water, as white whales from the high arctic. Thus body size cannot be a limiting factor for white whales *in winter*. It seems very likely that the limiting factor is the heat loss from newborn calves in the first few days after birth, before these have ingested enough milk to put on a blubber layer. In this connection the widespread movement of white whales into river estuaries in summer is probably significant. Birth occurs during the summer months. We found at Seal River, in western Hudson Bay, that newborn calves were to be found in July in the warm, brackish water of the estuary of this small river. Because of higher air temperatures, and because river discharges are greater, the coastal waters of Hudson Bay are warmer in summer than those of arctic Canada. This difference probably allows birth of smaller young in Hudson Bay.

The white whale's lack of success in any but an arctic or estuarine subarctic environment is shown by recent extralimital strandings or sightings in the Canadian Maritime Provinces (Fig. 15). The St. Lawrence population is resident and makes small migrations but, according to Vladykov (MS, 1944), extends to Baie des Chaleurs in spring when water temperatures are low and ice development is extensive. At this time, mainly during May, a number of extralimital records of white whales have recently been documented along the coasts of the Maritime Provinces, where they would naturally follow the Gaspé current around Cape Breton Island and the cold coastal Labrador current around Nova Scotia. They occasionally enter the Bay of Fundy (Fisher and Sergeant, 1955; Mairs and Scattergood, 1958). Here, however, they fail to survive and multiply, although the habitat is shallow, estuarine, and cool throughout the year. The porpoise *P. phocoena* is abundant in the Bay of Fundy and is evidently in some way better adapted to this environment.

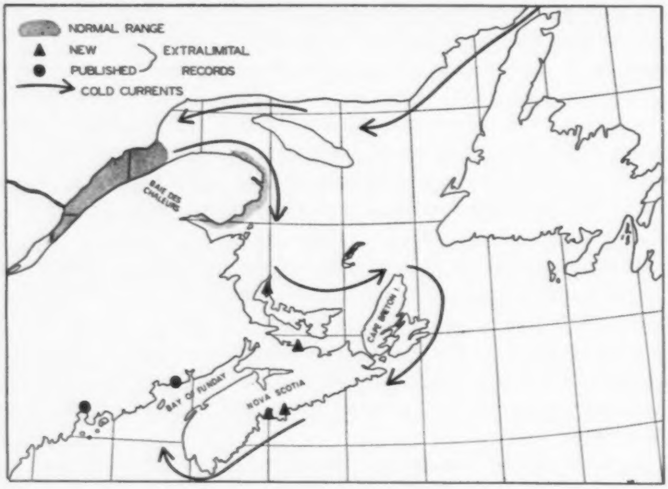


FIG. 15. Extralimital records of white whales in the Canadian Maritime Provinces and the spring range of St. Lawrence population.

Among the Delphinidae not adapted to residence in arctic waters is the killer whale *Orcinus orca* L. This species is well known to prey on sea mammals. It is recorded regularly in summer in Cumberland Sound and is reported to prey here on white whales. It also enters western Hudson Bay in August where it has been reported close to the herds of white whales. It is possible, therefore, that the white whale is restricted to the arctic or to subarctic estuaries (i.e., very shallow water) by predation from killer whales. The killer whale's blubber thickness does not allow it to calve in the Arctic, nor does its dorsal fin allow it to break ice. Thus it can penetrate arctic waters only in summer months. White whales therefore could be expected to enter very shallow water in the summer months and to penetrate oceanic and somewhat more southerly waters in winter. Both conditions are observed. As an alternative to competition, we may therefore postulate predation as a factor limiting white whales to the Arctic. Their inferior muscular development, as compared with a delphinid, may well lower their ability to avoid predation by killer whales.

Combining both relationships we suggest that in summer, white whales escape predation from killer whales by living in the arctic or in subarctic estuaries. Here possible competitors (e.g., *P. phocoena*) cannot follow them because they and especially their newborn young (also born in summer) are not thermally adapted to such cold water. However, the white whale's calf is big enough to survive birth in the cool estuarine water in summer. In winter, competition, or more likely predation, excludes white whales to icebound seas, their limit being approximately the border of the pack ice. The same type of argument could be developed for *Phocoena*, which, being smaller than

Delphinapterus, cannot penetrate to such cold water, but may itself be excluded to cool waters by other species of Delphinidae, and may avoid predation by an ability to enter shallower water than killer whales.

In the St. Lawrence estuary white whales are common, and no longer hunted. Their relations with other species could very readily be studied further at this site. In aquaria the muscular power or swimming speeds of *Delphinapterus* and *Phocoena* might be compared with those of, for instance, *Tursiops*.

ACKNOWLEDGMENTS

The measurements and other data of white whales from Canadian waters were collected by a number of other people in addition to the authors. They include the following personnel of the Arctic Biological Station: Dr J. G. Hunter, M. Riske, A. H. Peden, and W. Hoek at the Mackenzie Delta; Dr A. W. Mansfield and B. Beck at Cornwallis Island; and Dr L. Johnson of Somerset Island. Dr M. M. R. Freeman, now at Memorial University, Newfoundland, kindly made available data from white whales, which he collected at Grise Fjord, Ellesmere Island, in 1965–67. Mr John S. Vania, State of Alaska, Department of Fish and Game, Anchorage, kindly supplied unpublished manuscripts by James W. Brooks and Calvin J. Lensink from which we were able to extract length and other data for white whales from Bristol Bay. Dr A. V. Yablokov, Institute of Animal Morphology, Moscow, very kindly made available unpublished data on lengths and colours of white whales examined by members of that Institute in 1956–57 in the Soviet arctic. Dr E. D. Mitchell kindly criticised the manuscript.

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Modifications in Sampling Procedures as Applied to Studies on the Bacteria and Tubificid Oligochaetes Inhabiting Aquatic Sediments

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Brinkhurst, R. O., K. E. Chua, and E. Batoosingh. 1969. Modifications in sampling procedures as applied to studies on the bacteria and tubificid oligochaetes inhabiting aquatic sediments. J. Fish. Res. Bd. Canada 26: 2581–2593.

The KB corer has many advantages over other bottom-sampling devices including some other coring tubes. It may be used to obtain reliable estimates of the standing stock of benthic invertebrates inhabiting soft sediments and of their spatial distribution in lakes and rivers. It may also be used for studies of the vertical distribution of animals and bacteria, and could be used for a wide variety of studies on sediments. Multiple-unit versions have been built and operated, thus overcoming the size limitation imposed by the basic design. This paper communicates some results obtained using this sampler.

Brinkhurst, R. O., K. E. Chua, and E. Batoosingh. 1969. Modifications in sampling procedures as applied to studies on the bacteria and tubificid oligochaetes inhabiting aquatic sediments. J. Fish. Res. Bd. Canada 26: 2581–2593.

Le K.B. corer a de nombreux avantages sur les autres appareils d'échantillonnage des fonds y compris d'autres "coring tubes." Il peut être utilisé pour obtenir des estimations sûres des réserves présentes d'invertébrés benthiques habitant des sédiments mous ainsi que de leur distribution dans l'espace dans les lacs et les rivières. En outre, il peut être utilisé pour des études de la distribution verticale des animaux et des bactéries; cet appareil pourrait être employé pour une grande variété d'études de sédiments. Des versions à unités multiples ont été construites et ont fonctionné, surmontant ainsi la limitation de grandeur imposée par le modèle de base. Cet article donne quelques résultats obtenus à l'aide de cet appareil d'échantillonnage.

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INTRODUCTION

Some of the theoretical requirements of an apparatus designed to sample soft sediments and the animals and microbiota inhabiting them have been discussed by Brinkhurst (1967). It was concluded that the one instrument that answered most of these requirements was a coring device designed by Kajak (1965). The Jenkin corer described by Mortimer (1942) is probably every bit as effective, but it suffers from the disadvantage of having a large number of moving parts and is relatively more cumbersome to operate and (presumably) expensive to produce. It does have a closing mechanism that operates at the base of the core as well as the top, giving added security in sampling very liquid substrates, and probably overcoming the difficulty experienced with sandy sediments, which tend to drop out of a corer that closes

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only at the top. A later modification of the simple Kajak corer employs a simple bottom-closing device (see Kajak, 1969) and an intriguing method for accomplishing the same purpose was described by Burke (1969). The latter may be applicable to future models and may well enable a sampling tube to be used that has a larger diameter than those employed by the apparatus illustrated here.

Some other commercially available samplers (even corers) prove to bias estimates of the standing stock of invertebrates, especially tubificids, to such an extent that production estimates and other quantitative studies are subject to serious errors. The whole subject of sampling the benthos is reviewed by Kajak (1969), and so this paper will be restricted to a consideration of parallel trials of the Freshwater Biological Association Corer (Gilson or FBA corer), a modified Kajak sampler, and an Ekman supplied by Hydrobios Apparatebau G.m.b.H. Kiel. The versatility of the corer will be emphasized by presenting results of studies of the horizontal and vertical distribution of tubificids in the mud of Toronto Harbour. A further study on the vertical distribution of bacteria in sediments emphasized the uses of the corer, and introduces a new method of subsampling the bacterial population present in cores.

A modified version of the corer, known as the KB (Kajak-Brinkhurst) has been made available commercially through Wildlife Supply, Saginaw, Michigan, USA.

THE CORER

The corer is illustrated (Fig. 1) with a version of the release mechanism similar to that employed by Kajak (1965). The commercial instrument has a simple messenger-operated release to prevent even the slight chance of the self-closing mechanisms being released during descent. A modification of the system used by Rawson (1947) for automatic release of an Ekman grab may also be employed, as may the system used on the FBA corer.

In the illustrated version, the weight of the equipment suspended by a cable attached to the shackle (A) holds the two clamps (B) tight against the shaft of the closing mechanism (C). A rubber suction cup is attached to the end of the shaft (D). The shaft is held at the top of its free fall while the sampler is lowered to the sediment, but once the weight of the cable slackens, the two clamps release the shaft and the suction cup falls and covers the hole in the base plate, which is the same diameter as the corer. The last point is vitally important, as there must be no impediment to the flow of water through the apparatus. This is the major fault with the FBA corer, where water exits at the top of the open device during descent by passing through the narrow gap between a piston and the walls of the cylinder retainer and out through a number of small holes. The flow of water through the corer is greatly restricted.

The core tube (E) may be a thin walled metal tube or a thicker walled plastic tube. The maximum internal diameter seems to be about 5 cm as samples are often lost when wider tubes are used. The walls of the plastic tubes can be drilled at intervals, making narrow holes, which will allow a syringe to be inserted for the addition or subtraction of interstitial water, the microbiota, tracers, and the like. Permanent rubber inserts can be added, or the holes may simply be blocked with wax prior to use.

Additional weights may be added by hanging lead "doughnuts" around the tube supported by chain attached to the four lugs (F). This helps to keep the centre of gravity low, and prevents the apparatus from falling over sideways, as happens with the FBA corer where the weights are added at the top.

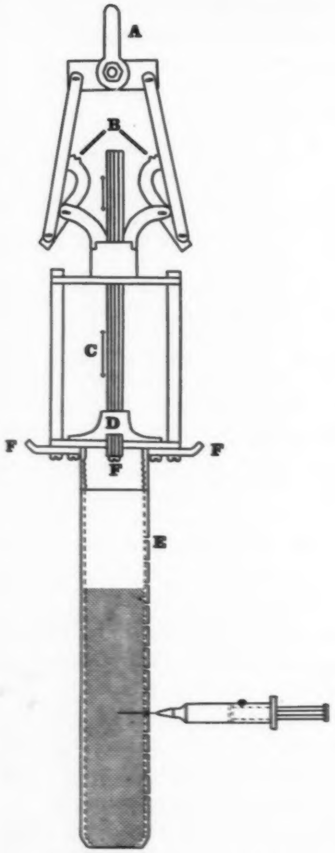

FIG. 1. The KB corer in the closed position. For further details see text.

Repeated samples can be obtained either by releasing cores into a receiving vessel or by adding a stopper to the base of the core tube, removing it from the sampler, and replacing it with another tube. Four- and seven-tube versions of the device have already been built and are being tested in other laboratories.

The Ekman grab used for these tests suffers from two deficiencies in design; the 15-cm-side square section is adequate for sampling dense populations but the box is only 16.5 cm high, and the upper doors do not open fully because they are impeded slightly by the bridle. It is superior to many commercial grabs in that it has supplementary lead weights, good springs (that can be unclipped from the jaws for easy setting), steel cables from the jaws to the release gear (rather than chain) but no screen in the upper opening. A tall version of the 15-cm Ekman with many such faults corrected is now marketed by Wildlife Supply, Saginaw, Michigan, USA.

The samples were sieved using screens with either a 0.45- or a 0.5-mm opening.

COMPARISON OF STANDING STOCK ESTIMATES PRODUCED BY CORERS AND THE EKMAN GRAB

The first parallel trial was run in Toronto Harbour using the FBA corer and the Ekman grab. The results are set out in Table 1A–D.

TABLE 1. Comparison of FBA corer with Ekman grab.

Set		Corer	Ekman
		Toronto Harbour	
A.	$\bar{x}$ Oligochaetes	25.8±11.6[a]	145.2±52.7
	$\bar{x}$ Chironomids	0.4±0	2.6±0.9
	$\bar{x}$ Others	0.9±1.2	0.1±0.1
B.	$\bar{x}$ Oligochaetes	41.9±18.7	232.6±88.6
	$\bar{x}$ Chironomids	5.2±2.5	13.8±5.9
	$\bar{x}$ Others	0.4±0	0.7±0.8
C.	$\bar{x}$ Oligochaetes	41.9±14.3	319.5±97.8
	$\bar{x}$ Chironomids	0.9±1.2	2.8±11.7
	$\bar{x}$ Others	0	0
D.	$\bar{x}$ Oligochaetes	112.7±36.5	426.8±173.6
	$\bar{x}$ Chironomids	1.7±1.4	8.6±6.2
	$\bar{x}$ Others	0	0
		Shallow stream near Dorset, Ont.	
E.	$\bar{x}$ Oligochaetes	87.0±34.3	126.2±35.6
	$\bar{x}$ Chironomids	46.8±9.0	32.0±6.2

[a] Numbers = $\bar{x}$ number of individuals per 0.01 m^2 ± 95% confidence limits.

The data represent four separate patterns of samples taken at different points in the harbour on various dates. Each single set of data shows the values obtained by averaging the population estimates obtained from 10 core samples and from 5 to 7 Ekman samples, expressed as numbers per 0.01 of a meter^2 to overcome the difficulty of comparing samples of different size. The data in Tables 1(set E) and 2 (set B, C, D) were obtained in the same manner; that in Table 2(set A) was based on four cores and a single Ekman sample.

A comparison between the standing stock estimates derived from cores and Ekman samples in each of the sets (A–D) of data in Table 1 clearly shows that the corer failed to collect as many oligochaetes as the Ekman by a factor of between five and seven. The estimates of abundance of chironomids derived from the corer are also less than those obtained with the Ekman, although the differences do not appear to be so dramatic.

The result may be thought to be caused by physical disturbance of the mud/water interface during the descent of the corer, and so a further trial was carried out in a small, organically polluted muddy stream near Dorset, Ont. Samples were collected using the utmost care to prevent any physical disturbance of the stream bed. The sampler was lowered very gently through the shallow water by hand, and the closing mechanisms were also operated manually. The water was sufficiently shallow and clear to allow a continuous watch on the mud/water interface of the sample area, and it was possible to collect all the samples without dislodging any appreciable amount of sediment from the area to be sampled. The result of this careful procedure (which is

TABLE 2. Comparison of KB corer with Ekman grab.

Set	Corer	Ekman
Port Credit Harbour, Ont.		
A. Prototype		
$\bar{x}$ Oligochaetes	340.6[a]	223.6
$\bar{x}$ Chironomids	5.5	3.7
$\bar{x}$ Others	104.9	100.7
Toronto Harbour		
B. Prototype		
$\bar{x}$ Oligochaetes	467.5±98.6	599.1±81.3
$\bar{x}$ Chironomids	11.6±6.1	16.4±2.7
$\bar{x}$ Others	8.3±7.7	0.9±0.4
C. Prototype		
$\bar{x}$ Oligochaetes	441.6±104.9	360.4±164.2
$\bar{x}$ Chironomids	12.7±4.6	7.8±2.8
$\bar{x}$ Others	3.3±2.4	4.5±1.8
D. Commercial version		
$\bar{x}$ Oligochaetes	749.4±396.9	697.4±173.0
$\bar{x}$ Chironomids	4.1±2.8	2.2±0.5
$\bar{x}$ Others	3.6±3.4	2.1±1.5

[a] Numbers = $\bar{x}$ number of individuals per 0.01 m^2 ± 95% confidence limits, where available.

not normally applicable in sampling through any but the shallowest water columns) was that the estimate of the abundance of oligochaetes obtained by using the corer was still substantially lower than that obtained with the Ekman, but that the value for chironomids was approximately the same in both instances (Table 1, set E).

The differences between the estimates of standing stock of oligochaetes obtained with these two devices are sufficiently large as to make it impossible to base quantitative studies on the use of the FBA corer, as there would seem to be little basis for believing that the use of the Ekman could magnify standing stock estimates.

Dr Z. Kajak of the Polish Academy of Sciences, Warsaw, Poland, had been working towards the production of a coring device at the same time as the senior author, and was kind enough to discuss his model and to provide sketches from which a prototype was built at Toronto. A few modifications were made, partially for convenience in local manufacture, but the basic design feature of overriding significance was retained. In this corer absolutely no impediment to the free flow of water through the corer is permitted, and hence no pressure builds up within the device.

In a preliminary trial of the prototype the standing stock estimates obtained using the new (KB) corer were slightly higher than those obtained using the Ekman (Table 2, set A), but this result could not be tested statistically because of the small number of samples obtained.

Two more sets of samples were taken with this new corer (Table 2, set B–C) and with the Ekman, and a third set (Table 2, set D) with the commercial version of the corer. The results of this trial are clearly more satisfactory than those using the FBA corer, and it is apparent by inspection that, with the high variation between estimates using any one sampler (itself an expression of the patchy distribution of the animals — see below), there is no sensible difference between the two sets of figures. The confidence limits for set D (the samples obtained with the commercial sampler) are exceedingly high, especially for the estimates using the corer. These samples were obtained by unskilled summer assistants using both sets of equipment for the first time, but, as the average standing stock estimates obtained are quite similar and the commercial sampler was not essentially different in fundamental design characteristics from the original KB prototype, no further trials were made at the time.

Both the Dorset stream and Toronto Harbour are grossly polluted with organic matter. The Tubificidae, especially *Tubifex tubifex* (Müller, 1776) and *Limnodrilus hoffmeisteri* Claparède, 1862, are the most abundant organisms present, with some chironomid larvae but few other benthic organisms. Amphipods may be found in shallow water in Toronto Harbour around Hanlan's Point (where the samples were collected for the most part) but they are seldom obtained in either Ekman samples or cores. A more diverse fauna was found in the samples from Port Credit, Ont. (Table 2, set A) at a time when the Credit River was in spate during the spring thaw.

Standing stock estimates of tubificids from Toronto Harbour, using mean values derived from all Ekman samples and the KB corer samples, ranged from 14,520 to 74,940/m^2 over various parts of the Harbour around Hanlan's Point, i.e., a median value of just less than 45,000/m^2 (or 9250 to 114,630 taking confidence limits into account, giving a median value of about 62,000). Chironomids are much scarcer, estimates ranging from 220 to 1640/m^2 with a median value of over 900/m^2 (or 170 to 1910, median value 1040). Using the largest individual sample values for oligochaetes and chironomids, the maximum values obtained were 230,970 and 3310 respectively.

In the Keating Channel, where the Don River enters Toronto Harbour, 12 samples were taken in four lines of three samples each (sides and middle at each point). Combining the data derived from all of these samples (taken with the KB corer) produced much larger population estimates. Data from the 12 samples on four transects combined yielded average values (with 95% confidence limits) of 2150 ± 1337 for *T. tubifex*, 870 ± 415 for *L. hoffmeisteri*, only 6 for *Peloscolex multisetosus*, and 3025 ± 1454 per 0.01 of a meter^2 for total oligochaetes.

Peloscolex multisetosus was scarce in samples from the two lower lines (nearest the Harbour) and was absent from the two upper sets of samples. These two observations, together with the absence of all other benthic organisms, give a clear indication of the much greater degree of pollution in the channel than in the Harbour. The average population levels of tubificids observed was greater than 300,000 ± 145,000/m², the highest individual total obtained indicating a population density of 777,000/m². Of the latter estimate, 755,688 were ***Limnodrilus hoffmeisteri***. The highest population density recorded for ***Tubifex tubifex*** was 177,744/m². These are the highest population estimates for tubificids that the senior author can recall.

SPATIAL DISTRIBUTION OF BENTHIC ORGANISMS IN SEDIMENT

Data derived from comparisons between sampling devices of two different sizes enables some estimate of the spatial distribution of the organisms to be made. The curve representing the relationship between sample means and variance for oligochaetes suggests that their distribution approximates the negative binomial (Fig. 2) as suggested by Alley and Anderson (1969).

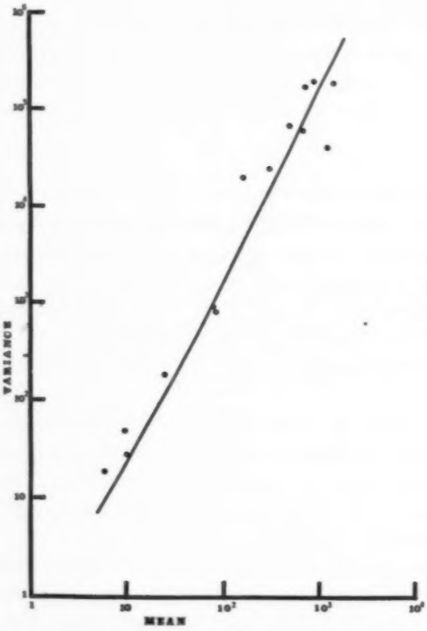

FIG. 2. Curve representing the relationship between sample means and variance for oligochaete population estimates in Toronto Harbour. Variance $= \bar{x} + \dfrac{\bar{x}^2}{7.24}$.

The curve for chironomids (in this instance mostly *Chironomus* sp.) also appears to fit the negative binomial, but the curve more closely approximates that of a Poisson distribution than that for oligochaetes (Fig. 3). The chironomids in the study by Alley and Anderson (1969) were said to be distributed according to the Poisson curve, but the fit to the points in their $\bar{x}$/variance plot is not good. The data in the present study is admittedly scanty, and the species concerned are almost certainly different from those encountered in the earlier study, and hence comparison of the two sets of data should be attempted with

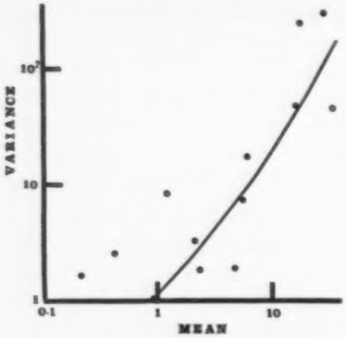

FIG. 3. Curve representing the relationship between sample means and variance for chironomid population estimates in Toronto Harbour. Variance = $\bar{x} + \frac{\bar{x}^2}{12.43}$.

caution, if at all. It would be more satisfactory if plots of individual chironomid species were available, as considerable variation in spatial patterns of distribution might be expected in this group, whereas the tubificids might be expected to show far less in view of their limited anatomical and behavioural diversity (although the last point is clearly an assumption rather than an established fact).

SAMPLE SIZE, NUMBER, AND VARIANCE

The high confidence limits in the tables of data indicate a serious lack of precision in benthic sampling, a factor usually ignored by benthologists. Samples are frequently duplicated in ecological studies on the benthos, but single-sample estimates are still commonly used. Much of the work on tubificids carried out by the senior author has been based on six samples per station wherever the sampling programme was designed and executed under his control.

There seems to be a general belief that, as conditions in the fine sediment in any reasonably deep water body must be about as uniform as can be found in nature, the truly benthic organisms must be randomly distributed. The extent to which recent studies show this to be fallacious only serves to enlarge the errors built into secondary production studies.

Two methods of reducing the size of the confidence limits of estimates of the abundance of nonrandomly distributed organisms are immediately apparent. To enlarge the size of the sampler would be one course, but that would lead to an increase in the time devoted to analyzing the sample, which is probably the most lengthy and costly part of the operation. Twelve samples with the KB corer approximate in volume to one with the "six-inch" Ekman. Hence the volume of sediment to be examined and the number of organisms to be counted is reduced significantly when using the corer rather than the Ekman. For the slight additional effort of taking a number of samples at each point, a far more precise estimate of the local abundance of benthic organisms can be obtained using the KB corer than the Ekman without significantly increasing the bulk of material to be examined. Even this labour may be reduced by utilizing a multi-headed corer, and such tools have now been designed and used in laboratories in Canada and the USA.

An estimate of the relative number of core samples per Ekman grab required for equally precise estimates by the two sampling methods is shown in Fig. 4, which shows that relatively few core samples may be required at any given spot. The number of worms in samples taken with the KB corer is low compared with the number collected by the Ekman (Fig. 5) for a wide range of population densities, and hence the saving in time and cost achieved by using the KB corer is quite significant.

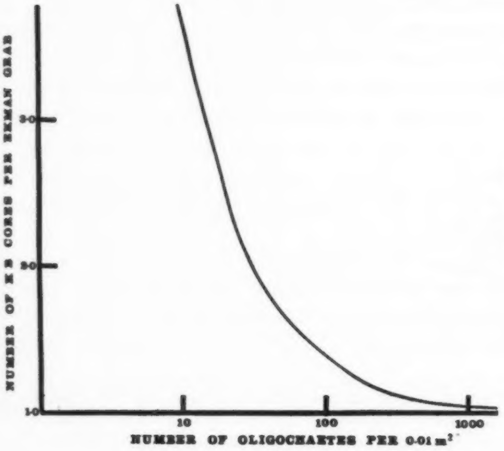

FIG. 4. Variation in the number of KB core samples required to obtain as accurate an estimate of oligochaete populations as that obtained by a single Ekman sample in relation to population density (expressed as mean number per 0.01 m²).

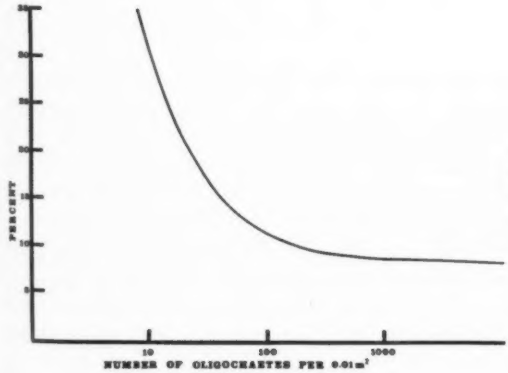

FIG. 5. Mean number of oligochaetes in a KB core sample expressed as a percentage of the mean count in an equally precise Ekman sample.

STUDIES ON VERTICAL DISTRIBUTION

The Oligochaeta

Brinkhurst and Kennedy (1965) determined the vertical distribution of tubificid species in core samples in a small, shallow, polluted stream. There was no evidence of spatial separation of the species present. During studies of the ecology of the three tubificid species present in Toronto Harbour the KB sampler was used to obtain a series of cores that were sliced at 2-cm intervals. Slices from 10 cores were pooled to produce each set of data, four such sets of data being obtained. The samples were sieved using a Wildlife Supply bucket with a screen (0.5-mm opening) set in the bottom.

The cores were not frozen in situ as it seems likely that any disturbance to the normal vertical distribution of the worms would occur during penetration of the sediments by the core tube, so that subsequent freezing would not be expected to affect the distribution observed.

As in the earlier determination of vertical distribution, most of the worms were found at or near the mud/water interface, but some were found as deep as 20 cm in the sediment (Table 3). By far the majority of the worms were found in the layer at which most feeding activity occurs, i.e., 0–6 cm. Again, there was no evidence of spatial separation of the three species. The difficulty in interpreting this result as a proof that the worms do not differ with regard to the depth at which they concentrate their feeding activity is that it is a static picture of what must be a dynamic situation. Worms presumably require access to the mud/water interface in order to respire, and if they spend more time at the surface respiring than at some preferred level in the sediment feeding, then at any given instant most will be found at the mud/water interface (assuming their movements are totally independent and there is no diurnal rhythm or coordinated activity). Attempts to follow the movements of tubificids within sediments using X-rays have thus far proved abortive as there is so little density difference between small worms full of sediment and the sediment itself.

Table 3. Vertical distribution of the three worm species in four samples of 10 cores each from Toronto Harbour.

	Limnodrilus hoffmeisteri				*Peloscolex multisetosus*				*Tubifex tubifex*			
Depth (*cm*)	(1)	(2)	(3)	(4)	(1)	(2)	(3)	(4)	(1)	(2)	(3)	(4)
0–2	150	192	296	153	6	18	34	45	93	204	129	179
2–4	45	51	92	68	4	9	0	10	27	33	79	49
4–6	25	19	43	22	0	1	2	2	24	6	34	7
6–8	20	14	21	13	2	2	1	2	18	4	11	8
8–10	9	11	15	8	1	1	1	2	2	3	15	2
10–12	2	4	0	2	0	0	0	1	0	1	0	0
12–14	0	1	0	0	0	0	0	0	0	1	0	1
14–20	0	1	0	1	0	0	0	0	0	0	0	0

THE BACTERIA

ZoBell (1938) and Sverdrup et al. (1946) described the vertical distribution of bacteria in cores obtained from beneath the sea. Ocevski (1966) reported on the vertical distribution of heterotrophic bacteria in a water column and to a depth of 18 cm into a freshwater sediment using a Jenkin sampler. The numbers of bacteria were quoted in terms of the number per milliliter of crude mud, and they were counted by the membrane filter method. No details were given of the method by which the volume of mud used was determined. In our laboratory, the KB corer was used to determine the relative abundance of heterotrophic aerobic bacteria at the mud/water interface and at various levels in the mud.

In the first method, the top 0.5 cm of mud were removed and the rest of the core was sliced at 2-cm intervals. Each of these subsamples was shaken with 200 ml of sterile water in a mason jar with glass beads for 5 min to disperse the mud particles and bacterial clumps. Duplicate 10-ml samples of the resulting suspension were then successively diluted with 90 ml of sterile water to give a final dilution factor of 10^6. The final 100-ml dilutions were filtered through a gridded millepore filter (0.45 μ pore size, 47 mm diam), which was then placed on pads soaked with nutrient broth and incubated for 5 days at room temperature before colony counts were made.

Dry weights of mud samples were obtained by drying 10-ml samples of the suspensions at 100 C. The resulting determinations are given in Fig. 6 for three separate cores.

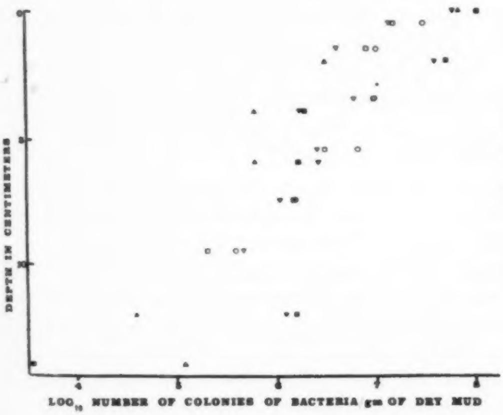

FIG. 6. The logarithm of the number of bacteria per gram of dry mud at different depths in six cores from Hanlan's Point, Toronto Harbour. Open symbols = results obtained from the first (indirect) method; closed symbols = results obtained using second (direct or syringe) method. Squares, circles, and triangles indicate results from individual cores.

A more direct method was also developed. One side of the acrylic core tube was perforated with openings at 1-cm intervals vertically, the openings being sealed with wax from a burning candle prior to sampling. The tube was sterilized with 70% alcohol and both ends were corked with neoprene stoppers until ready to be used in the field. When the core sample was obtained, the sampler was allowed to stand upright on a level surface, and the wax seals were removed. The area around each opening was washed with 70% alcohol, and then 1 ml of sterile distilled water from a vaccine bottle was introduced to the centre of the core. This was done by using a 10-ml, sterile, disposable, plastic syringe with 21 gauge needle and 1.5 inches length. The plunger was pumped back and forth and approximately 0.1 ml of the sample was withdrawn into the syringe, and the sample was injected into 9.9 ml of sterile distilled water in a test tube. Further dilutions of the samples were made in the laboratory. Culturing was done in duplicates by spreading 0.1 ml of each diluted sample in NA medium. The remainder of the samples were stored in their individual tubes, which were covered tightly with parafilm to prevent evaporation. After incubation at room temperature for 2 days the colony counts on the appropriate plates were determined by using a darkfield colony counter. Subsequently 0.5 ml of the samples from which the number of colonies was previously established was dried in pre-weighed planchets. They were dried in an oven at 70 C for 24 hr and then cooled to room temperature in a desiccator prior to weighing. The results obtained from three separate cores are also illustrated in Fig. 6.

In both sets of determinations, the heterotrophic aerobic bacteria were found to be most abundant at the mud/water interface but they decreased rapidly in abundance with depth into the sediment, hence confirming the findings reported by Ocevski (1966). There was also a reasonable agreement between the estimates of abundance produced by the two methods. It is felt that the syringe method was fully justified and, as it was the simplest method to use, it is recommended for future studies of this kind. The computation of numbers of bacteria relative to dry weight of mud is prefererd owing to the difficulty of obtaining accurate estimates of the volume of wet mud and differences in the water content of the mud samples.

DISCUSSION

The results demonstrate the versatility of the KB corer. The syringe method used to extract bacterial samples can clearly be used to extract water samples for chemical analysis or to introduce tracers to cores for studies on nutrition and feeding rates of microorganisms and benthos. Standing stock estimates of microorganisms and benthic invertebrates inhabiting soft sediments can also be determined with greater economy than when using the Ekman grab wherever the organisms are reasonably abundant.

Extremely high estimates of the abundance of tubificids in Toronto Harbour coincide with a low diversity of other benthic organisms and confirm the grossly polluted state of the locality. The vertical distribution of worms coincides with that of the heterotrophic aerobic bacteria, which may well form the bulk of the diet pf the worms. Any supposed interaction between the worms and the bacteria must, however, be a complex matter. The worms need access to the mud/water interface for respiration, and the number found at the surface in cores from Toronto Harbour probably reflects the prolonged period of respiration required for existence in such an environment. The faeces of the worms are deposited at the surface and these represent material ingested at some deeper level, and so the upper layer of mud is continually being circulated. Do these faeces prove to be a rich source of nutrients for the bacteria, or are they somewhat aerated in contact with the water? Does the abundance of bacteria at the mud/water interface depend on the fresh supply of material raining down through the water column or on the supply of finely divided faecal material continually being deposited on the surface of the mud from below? These questions must, for the moment, remain unaswered. The KB corer however, provides a simple versatile tool for use in studies of the interface and its fauna.

ACKNOWLEDGMENTS

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Acrocirridae n.fam. (Polychaeta Sedentaria)[1]

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Acrocirrus Grube and *Macrochaeta* Grube (Polychaeta Sedentaria), comprising at present 14 species, are removed from the Cirratulidae and placed in a new family. The descriptions of the type species of the genera and of five other species are materially improved. *Acrocirrus trisectus* and *Macrochaeta pege* are newly described; *Acrocirrus heterochaetus okotensis* Imajima is elevated to species rank. Diagnoses and records as well as keys for identification are given for all species of the family.

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INTRODUCTION

THE NEED TO CLASSIFY a species of *Macrochaeta* Grube from Puget Sound necessitated a study of the type species of the genus, *Macrochaeta clavicornis* (M. Sars), which was found to be poorly known. This led to an investigation of all species of the genus, as well as those of the closely allied genus *Acrocirrus* Grube. Until now these genera have been included among the Cirratulidae, although a long time ago a few authors pointed out differences separating them from other members of the family. In this paper a review of the scattered anatomical observations in the literature and a largely morphological study of specimens of most of the known species allow a clear delineation of characters setting these genera apart from the other polychaetes. On this basis it is suggested that *Acrocirrus* and *Macrochaeta* be removed from the Cirratulidae and placed into a new family. In addition to a discussion of the principal characters of the two constituting genera, some species are redescribed, and two species are described as new. Diagnoses, primary synonyms, and records are given for all known species. An effort has been made to locate the older types. In each instance the authority for the information is indicated.

The following abbreviations are used in the text: Allan Hancock Foundation, Los Angeles (AHF); Academy of Natural Sciences, Philadelphia (PA); British Museum, Natural History, London (BMNH); Hamburgisches Zoologisches Museum (HZM); Institut Royal des Sciences Naturelles de Belgique, Section Océanographie, "Belgica" Collection, Bruxelles (ISNB); Muséum National d'Histoire Naturelle (Laboratoire de Zoologie: Vers; Collection Fauvel), Paris (MNP); National Museum of Ireland, Dublin (NMI);

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Naturhistorisk Museum, Göteborg (NMG); Peabody Museum of Natural History, New Haven, Connecticut (PMNH); Station Marine d'Endoume, Université d'Aix-Marseille (SME); Stazione Zoologica, Naples (SZN); U.S. National Museum, Washington, D.C. (USNM); and Zoologisches Museum der Humboldt-Universität, Berlin (ZMB).

ACROCIRRIDAE n.fam.

Type genus *Acrocirrus* Grube, 1872

Diagnosis — Vermiform free-living Polychaeta Sedentaria with frontal grooved palps. Prostomium reduced, lying dorsally on first segment (peristomium); with eyes. Mouth opening subterminal. Proboscis unarmed, with a small buccal sack. Division of body into thorax and abdomen weakly indicated externally. One pair of large nephridia in the thorax, opening anteriorly. Gonads in abdomen. One pair of gills per segment, above notopodia, from segment two; usually four pairs. Epidermis with papillae. Setae usually from segment four, occasionally from segment five. Parapodial lobes of variable development, mostly very small. Several aciculae. Segmented spinose capillary notosetae; sometimes similar swimming setae in notopodia. Compound hooks with membranes below tips of blades as neurosetae, at least in anterior setigers.

Species can be recognized as members of the family from the shape of the notosetae and neurosetae alone. In addition, the family is distinguished from the Cirratulidae as restricted by Day (1967) by its frontal palps, by the prostomium that lies on the peristomium, by the arrangement of gills (somewhat resembling that of *Dodecaceria* among the Cirratulidae), by the parapodial lobes if present (including the papillae ventral of the neurosetae that resemble those of Orbiniidae), and by the presence of aciculae.

Presently, two genera can be distinguished: *Acrocirrus* Grube and *Macrochaeta* Grube. Neither is obviously the more primitive one. *Acrocirrus* has been chosen as the type genus of the family because its members are usually large enough to allow recognition of all diagnostic characters of the family.

Description — The habitus of the front end of a typical *Acrocirrus* species is shown in Fig. 1*a*. Usually one pair of eyes is clearly visible on the prostomium, and can be accompanied by one or two pairs of small eyespots. Whether the palps are of prostomial or peristomial origin is not definitely known. Among the authors who recognized the location of the prostomium correctly, von Marenzeller (1879), Mesnil (1899), and Eliason (1962) considered the palps to be prostomial, whereas Grube (1860, 1873), McIntosh (1923), and Moore (1923) believed them to be peristomial. The palps appear to insert on the prostomium in *A. heterochaetus* treated below, but similar insertion is less obvious in *A. trisectus* n.sp. Marion and Bobretzky (1875) who studied living specimens of *A. frontifilis* observed that the longitudinal ciliated groove on the underside of the palps carries food particles to the mouth. Therefore, *M. clavicornis* was found to be a selective deposit feeder on coarse sediment (p. 2612). Below and slightly outward from the insertion of the palps (i.e., below the end of the ciliated groove of the palps) is a pair of thick-walled, pillowlike elevations that are clearly a part of the peristomium (Fig. 1*b*; see also *M. papillosa*). Their surface is without epidermal papillae. Okuda (1934) has observed in *A. uchidai* that the frontal slits of these elevations are ciliated.

The proboscis when everted (seen by me once each in *A. crassifilis* (Fig. 1*b*), *M. clavicornis*, and *M. papillosa*) is little more than a large ventral lip. Okuda (1934) has reported a feebly developed buccal mass for *A. uchidai*. The first segment can be fused ventrally with the second one. This segment, in turn, can appear united ventrally with the third segment, as in some species of *Acrocirrus*. In *M. papillosa*, the first and the second segments are dorsally reduced. Marion and Bobretzky (1875) have found that the dorsal appendages on the segments following the first segment are gills. Their number is four pairs, except in one or two *Macrochaeta* species. The second pair always inserts more dorsally than the others (Fig. 1*a*).

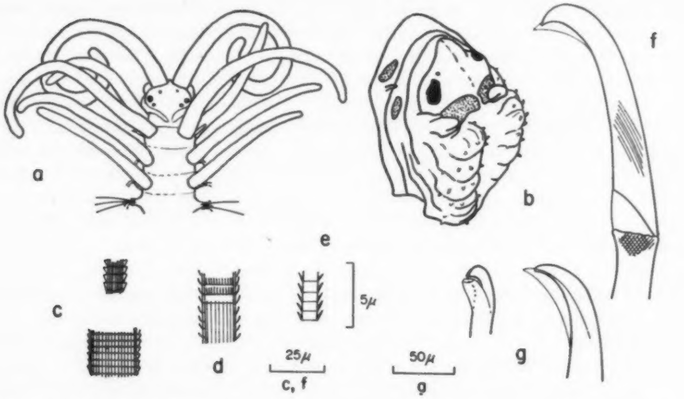

FIG. 1. (*a*) *Acrocirrus frontifilis*, dorsal view of anterior end (modified from Marion and Bobretzky, 1875); (*b*) *A. crassifilis*, anterior end with everted proboscis from the right; all appendages lost (scars indicated by stippling); (*c*) *A. crassifilis*, middle and distal section of a capillary seta; (*d*) *A. heterochaetus* (from Alaska), middle section of a capillary seta; (*e*) *Macrochaeta pege*, distal section of a capillary seta; (*f*) *M. clavicornis* (from Ireland), distal part of a thoracic compound hook; (*g*) *A. crassifilis*, tip of blade of a compound hook, en face and almost en profile.

In all species of *Acrocirrus*, and many species of *Macrochaeta*, the setation indicates two body regions, a thorax extending to the 11th–13th segments, and an abdomen. Usually the cutting edges of the blades of the thoracic compound setae face rearward; the abdominal ones face forward. In all *Acrocirrus* species, the first abdominal segment is segment 13. The extent of the thorax as indicated by the setae is variable within *M. papillosa* and probably also *M. clavicornis*, but is apparently a specific character in *M. pege* n.sp. The tendency in the family to a constant number of gills and of segments forming the thorax is noteworthy.

In the three species of *Acrocirrus* studied by Okuda (1934), one pair of large U-shaped nephridia lies on the sides of the gut. The funnels are in the third segment in *A. uchidai*. The canals reach to the 10th–13th segments, varying among the species, and return to the second segment. Openings were not found upon dissection by Okuda, nor by Marion and Bobretzky (1875) who studied the same organ in the living *A. frontifilis*, which is transparent while the organism is alive. In the latter species, the nephridia extend from the 4th to the 11th segments. Nephridia have not yet been studied in *Macrochaeta*.

Below the gill on the third segment is a cirruslike papilla in all species of *Acrocirrus*; it is visible in many species of *Macrochaeta* although the epidermal papillae may conceal it in other species. It is hollow in *A. crassifilis* and *M. clavicornis*, the two species studied by me in this respect. This papilla is perhaps the nephridiopore. Schlieper (1927) has pointed out, however, that the openings of the large nephridia of *Brada* Stimpson (Flabelligeridae) are in the head region, so that the two pairs of conspicuous ventral papillae on the fourth and fifth setigers of species of that genus are not nephridiopores.

In the three *Acrocirrus* species studied by Okuda (1934), the gonads start in the first or second abdominal segment and occur in the subsequent 17–25 segments. Also in a specimen of *A. frontifilis* from Morocco (now in Paris, p. 2600) and one of *A. heterochaetus* from Alaska, some eggs were floating in the coelomic cavity of the first and second abdominal setigers, respectively. In a specimen of *M. clavicornis* from the Kattegat, eggs occur from segment 13, the first abdominal segment of the species.

Pygidial appendages seem to be absent. There are small papillae in some species (see *M. helgolandica* Friedrich, p. 2615; for *M. sexoculata*, cf. Webster and Benedict, 1887).

The setae are very characteristic for the family: As seen in *Acrocirrus* species the notosetae have striated cores and segmented mantles, features that are most distinct in their middle and distal sections (Fig. 1*c*). When the "joints" of notosetae are long, as in *A. heterochaetus*, spines can be clearly seen (Fig. 1*d*; see also Hartman, 1948, fig. 11*c*). Long swimming setae replacing the ordinary notosetae have the same structure as normal notosetae. In damaged swimming setae of *A. frontifilis* from the Arabian Sea where the mantle has sprung off, the striated core is likewise clearly segmented. This may be true for all capillary setae. Whereas the core is poorly visible in *Macrochaeta* setae because of their small diameter, spines on their fairly long "joints" can be well made out, particularly distally on unworn bristles (Fig. 1*e*). The notosetae are different from the larval setae of Spionidae and Sabellariidae from the summer plankton of Friday Harbor, Washington (contrary to the statement by Caullery and Mesnil, 1898).

The neurosetae of all species, at least in the thorax, are compound hooks (Fig. 1*f*). The blade of such a seta is connected with the shaft by a hyaline membrane. Another membrane arises below the tip of the blade (Fig. 1*f*, *g*). The compound setae of Sigalionidae, said to be similar to these setae, are only superficially so. Other neurosetae of the Acrocirridae are clearly related to the compound hooks (as some neurosetae of *M. helgolandica*), or are heavy acicular hooks. In some species of *Acrocirrus* a heavy acicular hook with a widened head replaces the neurosetae on the 14th (second abdominal) segment. The aciculae, which are present dorsally and ventrally, are simple needles without segmented mantles. I found these in both rami of *A. crassifilis*, *M. clavicornis*, and *M. pege*; Okuda (1934) did not record notopodial aciculae for three Japanese *Acrocirrus* species. The aciculae alternate usually with the hooks; the notopodia of *M. pege* have two to four aciculae but only one or two setae.

Discussion — On the basis of the known characters of the new family it is clear that it is related to the Sedentaria that possess a buccal organ, in particular the Cirratulidae in the now narrower sense, the Ctenodrilidae, and the Terebellomorpha (Terebellida sensu Dales, 1962). As suggested by Schlieper (1927), the Flabelligeridae should be added to these families since they have a buccal organ (a fact not considered by Dales). Further, they have a heartbody, large anterior nephridia, and chlorocruorin as blood pigment, among other features. Mesnil (1899) had already noted this and had called the group of families the Cirratulimorpha.

Until now, *Acrocirrus* and *Macrochaeta* have been included among the Cirratulidae, the former genus since the description of the species that was to become the type of the genus and the latter genus since Langerhans (1881). The Acrocirridae, however,, seem to have fewer characters in common with the Cirratulidae than they have features of their own. Actually, one could find more characters the Acrocirridae share with the Flabelligeridae than with the Cirratulidae. Mesnil (1899) recognized this when he considered *Acrocirrus* and *Macrochaeta* as the next relatives of the Flabelligeridae among the Cirratulidae; now, one could add the buccal sack, presence of aciculae, and possibly the segmented mantle of the notosetae (see Schlieper, 1927, for the Flabelligeridae) to Mesnil's list. It seems, however, that an evaluation of the relative degrees of similarity should be preceded by an anatomical study of *Acrocirrus* spp., with emphasis on the innervation of the palps (cf. Orrhage, 1966), the structure of the buccal sack, the presence or absence of a heartbody, the blood pigment, and the anatomy of the epidermal papillae. The development is apparently unknown in both families.

Distribution — The distribution of the family is worldwide. *Acrocirrus* spp. are now known from the eastern warm-temperate North Atlantic Ocean, including the Mediterranean Sea, the North Pacific Ocean (here, six out of eight species occur), and the North Indian Ocean. The four species that have compound hooks in all setigers (a character that is presumably primitive) are North Pacific forms. *Macrochaeta* spp. have been found in the North Atlantic Ocean (including the deep sea at 5000 m), the cool-temperate North Pacific Ocean, and near the Antarctic continent.

Acrocirrus Grube, 1872

Type species *Acrocirrus frontifilis* (Grube, 1860)

Diagnosis — Acrocirridae with palps originating closely together, usually at least as long as the first 10 segments. Four pairs of gills. A conspicuous papilla always present on the third segment below the gill. Segments short but well marked, at least anteriorly behind the gill-bearing region. Thorax always comprising the first 12 segments; occasionally with conspicuous parapodial lobes. A row of papillae ventral to the neurosetae in thorax and abdomen. Body surface almost smooth, with a few, small epidermal papillae; papillae sometimes arranged in rows. Grown specimens of all species several centimeters long, with more than 50 segments.

Remarks — The most obvious diagnostic characters within the genus are the kinds of the neurosetae and their distribution over the body regions. Other characters are perhaps the development of parapodia and the presence or absence of a large papilla on the second segment, above the gill. Fusion of the second and third segments as reported by several authors, or absence thereof, is difficult to recognize when the specimens have a wrinkled epidermis due to preservation, and is not considered here.

The known species belong to three groups: The first group comprises the species with compound setae throughout (*A. crassifilis*, *A. muroranensis*, *A. uchidai*, and *A. validus*), all from the North Pacific Ocean. On the basis of the occurrence of only compound neurosetae, the group is most similar to *Macrochaeta*. The second group consists of *A. frontifilis* and *A. heterochaetus*, in which segment 14 is modified and equipped with a heavy neuropodial acicular hook. The members of the third group, *A. okotensis* and *A. trisectus*, have simple hooks in at least some abdominal setigers, but *A. okotensis* relates to the second group by the heavy hook on segment 14.

KEY

1	All setigers, or all except 11th, with compound hooks	2
	Many setigers past the 10th or 11th with simple hooks	7
2	A heavy acicular hook in each parapodium on the 11th setiger	3
	Only compound hooks	4
3	With notopodial cirri (Fig. 2*b*, *d*)	*A. frontifilis*
	Without notopodial cirri (Fig. 4*b*)	*A. heterochaetus*
4	Neurosetae from fourth gill-bearing segment	*A. crassifilis*
	Neurosetae from third gill-bearing segment	5
5	Notosetae from second setiger	*A. uchidai*
	Notosetae from third setiger	6
6	Upper face of prostomium smooth	*A. muroranensis*
	Upper face of prostomium ridged	*A. validus*
7	A heavy acicular hook (similar to Fig. 2*c*) in each parapodium on the 11th setiger, thereafter simple bent hooks each with small tooth below the tip	*A. okotensis*
	Simple bent hooks with smooth tips from 10th through 38th setigers	*A. trisectus*

Acrocirrus frontifilis (Grube)

Fig. 1*a*, 2

Heterocirrus frontifilis Grube, 1860, p. 89, 1861, p. 145; Marion and Bobretzky, 1875, p. 64.

Acrocirrus frontifilis Grube 1873, p. 65; LoBianco, 1893, p. 5; Fauvel, 1937, p. 32; Pérès, 1954, p. 135; Bellan, MS, 1964, p. 121.

? *Acrocirrus frontifilis* Fauvel, 1927, p. 104.

Macrochaeta clavicornis Fauvel, 1936a, p. 75.

Types — The holotype, which had been deposited in the ZMB, was destroyed during the Second World War (Dr G. Hartwich, Humboldt-University, Berlin, personal communication). The type locality is near the town of Cres (Cherso), Yugoslavia, on stones (Grube, 1860) and, more specifically, in about 4-m depth among *Melobesia* (Grube, 1861, p. 77, 81).

Diagnosis — An *Acrocirrus* species with notopodial cirri and neuropodial lobes prominent in the thorax. Neurosetae from segment 4, notosetae from segment 6. An acicular hook replaces the compound neurosetae on segment 14. A conspicuous papilla above the first gill.

The species is distinguished from *A. heterochaetus* by its prominent thoracic notopodia. It shares with this species the thoracic neuropodial lobes and the presence of compound neurosetae on all setigers except the 11th where an acicular hook is found.

Material studied — Mediterranean Sea, off Naples, Secca della Gajola. Collected and identified as *A. frontifilis* by S. LoBianco, November 1894 (an apparently unrecorded specimen, from one of the sites of the records by LoBianco, 1893). This specimen is the only one at the SZN; it is the best representative of the species I have seen.

Mediterranean Sea, off Marseille, station 202 of SME, Baie du Praolo, among *Peyssonelia polymorpha*, 35 m. Collected and identified before 1954 by J. M. Pérès (fide Dr G. Bellan, Marseille, personal communication; this is probably the specimen on which the record by Pérès 1954, was based). SME, one specimen. Bellan (MS, 1964) has recorded the species from "*Amphioxus* sand" near Marseille and believes (personal communication) that his specimens agreed with the description by Pérès (1954).

New record: Adriatic Sea, near Rovinj (Rovigno d'Istria), northern Yugoslavia. Collected and identified as *A. frontifilis* by K. Heider, April 1913. ZMB, No. 7664, two specimens.

New record: North Atlantic Ocean, off Morocco (Fauvel, 1936a, as *Macrochaeta clavicornis*). BMNH, No. 1928:4:26:719/20 (one specimen). MNP, two specimens. Fauvel (1936a) reported only one head but both samples are labelled as coming from station 57; the vial from the Fauvel collection also contained a *Dodecaceria* specimen, not recorded previously for this station.

New record: Arabian Sea, from eastern Gulf of Oman. International Indian Ocean Expedition, "Anton Bruun" station 269 C, 23°35′N, 58°49′E, 121–124 m, December 3, 1963. To be deposited in USNM. One specimen.

Redescription — The species is incompletely known. The redescription is largely based on the material from Rovinj, which is close to the type locality. An anterior fragment of 21 setigers is about 5 mm long and about 0.75 mm wide (without parapodia). A posterior fragment, which may be of the same individual, has about 85 setigers. A smaller, complete specimen has about 65 setigers. All palps and gills have fallen off. Palps in the vial are almost 4 mm long and 0.2 mm thick. Gills in the vial are up to 2 mm long and 0.2 mm thick. (Large specimens can be 8 cm long, LoBianco, 1893.)

The prostomium (Fig. 2*a*) lies dorsally on the first segment and is clearly demarcated laterally and posteriorly by a yellow margin. Two large eyes are accompanied by two small eyespots slightly below the epidermis. A transverse line crosses the surface of the prostomium. In the middle of the upper portion thus divided off is a longitudinal ridge. Anteriorly, below the prostomium, is a pair of cushionlike elevations.

The first segment reaches as a furrow to the dorsal side and separates the prostomium from the second segment laterally and dorsally except in the middorsal line. It bears laterally and ventrally a few scattered papillae. The second segment (first gill-bearing segment) has a papilla, situated dorsally and anteriorly of the gill scar, that is clearly more conspicuous than the scattered epidermal papillae. The third segment has a dorsally displaced gill and bears an unusually long papilla just below the gill scar. The fourth segment is the first setiger, which — like the next segment — has neuropodia only. In both segments, a vertical row of papillae extends between each neuropodium and the gill scar. The following segments have similar rows between the notopodia and neuropodia. Notosetae are present from the sixth segment (third setiger). All specimens have four pairs of gills, including the individual from Naples, contrary to the statement by LoBianco (1893) about other material from the same site.

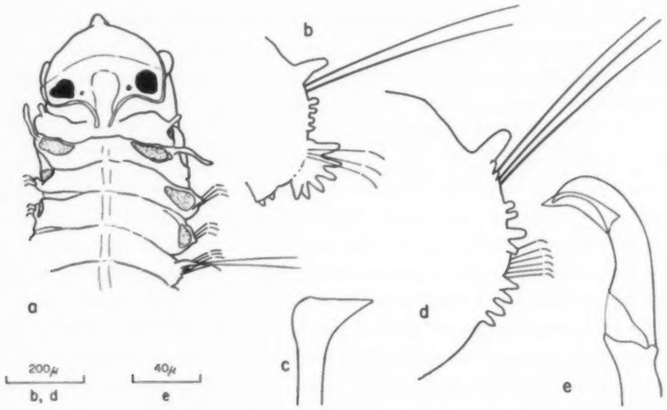

FIG. 2. *Acrocirrus frontifilis* (*a*, *b*, *d*, *e*, from Rovinj: *c* from Morocco, in BMNH): (*a*) dorsal view of anterior end, slightly from the right; all appendages lost; (*b*) anterior view of fifth parapodium, the papillae ventral of the parapodium partially cut off; (*c*) simple hook from 11th parapodium (segment 14); (*d*) posterior view of 19th parapodium; and (*e*) compound hook from 19th parapodium.

The parapodia in the anterior and middle regions of the body insert in the middle of the segments. Posteriorly, the insertion moves rearward and the anterior part of each segment becomes constricted, thus giving the body outline a jagged shape when seen from above. The parapodia insert slightly below the middle of the body. They are very prominent, particularly from the 3rd through the 10th setigers because of cirri and papillae in the notopodia and digitate postsetal lobes in the neuropodia (Fig. 2*b*; one or two small papillae are present at the origins of the notosetae in the last thoracic setigers, similar to those shown in Fig. 2*d*). On segment 14, the neuropodium is reduced and the compound setae are replaced by a large, hyaline or yellow, acicular hook (Fig. 2*c*). This hook can point forward to a papillated ridge (or possibly be hidden below?), as depicted for *A. heterochaetus* (p. 2605), or be directed sideward. Posterior to this segment, the neuropodial lobes are absent (Fig. 2*d*), but the parapodial structures still are larger than in the other *Acrocirrus* species.

The two to three notosetae of a ramus are up to 1 mm long, and thus are almost twice as long as the body is wide posteriorly. They have a striated core surrounded by transverse rings of small spines; the spines are less evident proximally, as usual also in other *Acrocirrus* species. The joints of the setae are 1–1.5 μ long. Two to four neuropodial compound hooks, with membranes below the tips, are found in the anterior part of the body. Their cutting edges face rearward through segment 12, and forward on the 13th and from the 15th segments onward. From the 13th segment the blades become relatively shorter (Fig. 2*e*). The number of compound hooks is three to five in the posterior region.

There are small, scattered epidermal papillae. The anus is terminal, or slightly displaced dorsally. The color of preserved animals is yellow brown.

Remarks on new records — The larger of the two specimens from Morocco, now at MNP, is an anterior fragment of a mature female with 23 segments. The animal is noteworthy because of a longitudinal mid-dorsal furrow dividing the third segment (the midline in Fig. 2*a* indicates the blood vessel seen through the epidermis). The segments of the specimen become wider from about segment 11. Starting from segment 13 the body is densely packed with polygonal eggs, up to 100 × 130 μ in size. At segment 19, the diameter of the body is almost 1 mm, about twice that of the anterior segments. From the 6th through about the 10th segments one to two long notosetae occur. By segment 14, they have become somewhat longer, and their number has increased to almost one dozen forming a fan of swimming setae. Subsequently, a ramus has up to 15 such setae, together with four to six neuropodial hooks. (Also one of the anterior fragments from Alexandria has fans of notosetae in about the same arrangement, of which the dorsal-most ones are as long as the notosetae on the first segments, whereas the lower ones are about half as long.) The other animals from Morocco are not well enough preserved to allow any detailed study of the anterior end.

A mature male (broken, about 85 segments) from the northern Arabian Sea very probably belongs to this species. Thoracic parapodia consist of a notopodial cirrus (Fig. 2*b*), interramal papillae, and a neuropodial postsetal lobe with three or four fingerlike papillae. The neuropodial lobes are only about as well developed as depicted for *A. heterochaetus* (p. 2605). However, to judge from that species, the apparent degree of development of these lobes may be subject to the condition of preserved material. Notopodial swimming setae are almost 1.5 mm long and occur on about 23 segments, anteriorly in the same arrangement as described above for Moroccan material. A clear difference between this specimen and the Adriatic material is that the blades of the thoracic neurosetae are about one and one-half times as long, relative to their width, as those of the Adriatic material.

Discussion — The opinion that the material described here is *A. frontifilis* (Grube) rests on the presence of large parapodia (an uncommon character in the genus) and the fact that all Mediterranean and Atlantic records of the genus belong to one species. It must be noted, however, that Fauvel (1927) stated that *A. frontifilis* had acicular neurosetae from the 11th setiger (i.e., segment 14) onwards. The basis of this statement is not clear in view of the records quoted by him. I consider the text as an erroneous interpretation of the description by Marion and Bobretzky (1875).

Distribution — *Acrocirrus frontifilis* as described here occurs in the northern Adriatic Sea and in the Mediterranean Sea off Alexandria, Naples, and Marseille. Another record comes from the French Mediterranean coast near the Spanish border (Banyuls-sur-mer; found and identified by Pruvot, see Laubier and Paris, 1962); the material is lost according to Dr L. Laubier, Banyuls (personal communication). The species has been further found off Morocco and in the northern Arabian Sea.

Acrocirrus validus von Marenzeller

Acrocirrus validus von Marenzeller 1879, p. 148; Annenkova, 1938, p. 183; Imajima and Hartman, 1964, p. 297 (with other Japanese records).

Acrocirrus varidus (sic) Okuda, 1934, p. 206.

Not *Acrocirrus validus* Ehlers, 1907, p. 23 (see under *A. trisectus* n.sp.).

The diagnosis is based on the descriptions by von Marenzeller (1879) and Okuda (1934).

Diagnosis — An *Acrocirrus* species with neurosetae from segment 4. Notosetae from segment 6. Only compound neurosetae. The upper face of the prostomium with characteristic ridges.

The species is distinguished from the other species with compound neurosetae throughout and starting on segment 4, by the absence of a conspicuous papilla on segment 2 above the gill, and the presence of notosetae from segment 6 (compared with *A. uchidai*); and by the compactness of the body and the ridged upper face of the prostomium and peristomium (compared with *A. muroranensis*).

Material studied — None.

Remarks — From von Marenzeller's description and the study of *A. frontifilis* material, I interpret the large crescentic ridges on the upper face of the prostomium (emphasized by Okuda, 1934) as associated with the border between the prostomium and the first segment, if they are not simply the first segment itself (compare Fig. 2*a* with Okuda, 1934, fig. 8*a*).

Distribution — Pacific and Sea of Japan coasts of Honshu; Japanese Inland Sea; mainland coast of Sea of Japan.

Acrocirrus crassifilis Moore

Fig. 1*b*, *c*, *g*, 3

Acrocirrus crassifilis Moore, 1923, p. 188; Hartman, 1961, p. 31, 1963, p. 49.

Acrocirrus? crassifilis Hartman, 1955, p. 47, 168.

Diagnosis — An *Acrocirrus* species with neurosetae normally from segment 5; notosetae from segment 6. Only compound neurosetae. Anteriorly, epidermal papillae arranged in rows.

The species is distinguished from the other species with only compound neurosetae primarily by the start of setae on segment 5 rather than 4.

Material studied — Off Redondo Beach, California (record by Hartman, 1955, 1961, 1963). Some specimens were inspected at the Allan Hancock Foundation, and three animals kindly lent by Dr O. Hartman were closely studied.

Additions to the original description — The prostomium (Fig. 1*b*, 3*a*) has a longitudinal ridge in the same position as *A. frontifilis*. The first segment is coalesced ventrally with the second segment. Scattered, fairly large papillae are conspicuous on the first as well as on the following segments where they are clearly arranged in rows. The peculiar mid-dorsal intersegmental pores mentioned by Moore for the type are very apparent also on these animals. The body wall is very thin and transparent posteriorly, more so than in other *Acrocirrus* species.

No animal of the present material reaches quite the length of 77 mm as did Moore's animal. All palps of the material from AHF had fallen off, but judging from the type specimen (Moore, 1923) the species has unusually long palps and gills.

Notosetae and neurosetae arise directly from the body wall (Fig. 3*b*); the interramal papillae, as well as those above the notopodia, are secretory, as shown by glandular tissue below. As already noted by Moore, neurosetae start from the fifth segment. However, in one out of the nine specimens studied in this respect, neuropodial tubercles, with a seta in one of them,

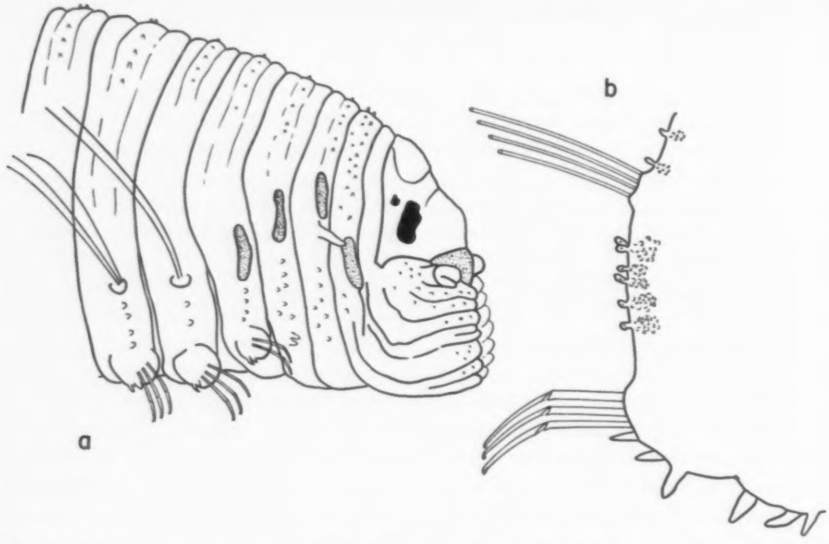

FIG. 3. *Acrocirrus crassifilis*: (*a*) anterior end from the right side, all appendages lost; (*b*) anterior view of eighth parapodium.

are present on the fourth segment on both sides. Through the 12th segment, the cutting edges of the blades of the hooks face rearward; thereafter, the direction is forward. Notosetae occur from the sixth segment. They are up to 2 mm long; in the middle and posterior sections of the body, they are longer than the body is wide. Their rows of very fine spines are about 1.5 μ apart.

Distribution — Off California, slope depths.

Acrocirrus uchidai Okuda

Acrocirrus uchidai Okuda, 1934, p. 198.

Diagnosis — An *Acrocirrus* species with neurosetae from segment 4. Notosetae from segment 5. Only compound neurosetae. A conspicuous papilla above the first gill.

The species is primarily distinguishable from the other species that have compound neurosetae throughout that start from segment 4 (*A. validus* and *A. muroranensis*), by the papilla on segment 2, above the gill, and by the notosetae on segment 5.

Material studied — None.

Distribution — Pacific coast of Hokkaido, Japan.

Acrocirrus muroranensis Okuda

Acrocirrus muroranensis Okuda, 1934, p. 202.

Diagnosis — A slender *Acrocirrus* species with neurosetae from segment 4. Notosetae from segment 6. Only compound neurosetae. The upper face of the prostomium without ridges.

This species is primarily distinguishable from the other species that have compound neurosetae throughout that start from segment 4, by the absence of a papilla on segment 2, by the occurrence of notosetae from segment 6 (compared with *A. uchidai*), and by its slenderness and the smoothness of the upper face of the prostomium and peristomium (compared with *A. validus*). Probably a more important character separating *A. muroranensis* from *A. validus* is the shorter extension of the thoracic nephridia in the former (Okuda, 1934).

Material studied — None.

Distribution — Pacific coast of Hokkaido, Japan.

Acrocirrus heterochaetus Annenkova

Fig. 1*d*, 4

Acrocirrus heterochaetus Annenkova, 1934, p. 326, 1938, p. 183; Hartman, 1948, p. 38; Ushakov, 1955, p. 304.

Diagnosis — An *Acrocirrus* species with prominent neuropodial lobes in the thorax. Neurosetae from segment 4. Notosetae from segment 5 or 6. An acicular hook, with a broad head, replaces the compound neurosetae on segment 14. A large papilla above the first gill.

This species shares with *A. frontifilis* prominent thoracic neuropodial lobes and the presence of compound neurosetae throughout (except in segment 14), but is distinguished from that species by the absence of notopodial cirri in the thorax.

Material studied — Coast of Alaska: Two broken, moderately well-preserved specimens from USNM labelled "USNM 22819, Station 24-40," identified and described by Hartman (1948; in that paper, one specimen each is reported for two stations, one of which is the above-numbered one).

New record: Eastern North Pacific Ocean, off Palos Verdes Estates, southwest of Los Angeles, California. "Velero" Station 1446-42, along rocky shore, from surface of kelp. Collected by O. Hartman, February 13, 1942. AHF, one specimen.

Remarks — In the Alaskan material, the pear-shaped longitudinal ridge on the prostomium known from other species (cf. Fig. 2*a*) is very low and barely visible. The first segment is laterally well separated from the following one except ventrally (Fig. 4*a*). The second and third segments are fused dorsally as well as ventrally. In the larger animal from Alaska, a longitudinal mid-dorsal furrow (cf. *A. frontifilis*, p. 2601) was observed. In both specimens, a conspicuous

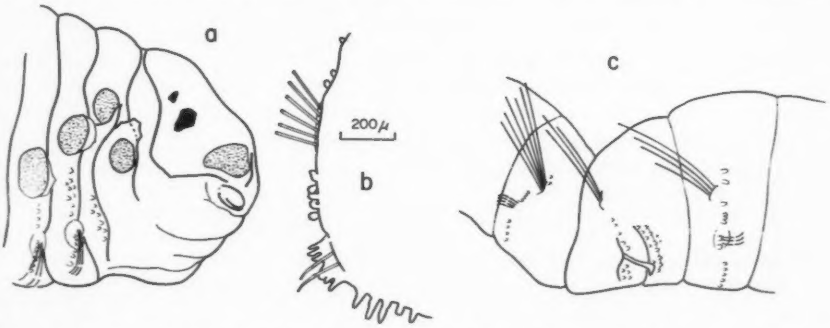

FIG. 4. *Acrocirrus heterochaetus* (*a*, *c*, from Alaska; *b* from California): (*a*) anterior end from the right side; all appendages lost; (*b*) anterior view of sixth parapodium; (*c*) 13th to 15th segments, somewhat bent, from the right side (slightly schematic).

papilla is present above the first gill. Neuropodia start on the fourth segment. Very tiny, scattered epidermal papillae occur. Larger papillae are associated with the parapodia (Fig. 4*a*) and include a row of up to half a dozen widely spaced papillae above the notopodia.

The cutting edges of the blades of the neurosetae face rearward through segment 12. Those of segment 13 and from segment 15 onward face forward; their blades are shorter than those of the thorax. The acicular hook on segment 14 originates from a fold posterior to the vertical row of papillae extending from the notopodium to the position usually held by the neurosetae (Fig. 4*c*). The hook reaches across this row and may either be hidden below an anterior, papillated fold, or directed laterally. Notosetae start on segment 6 in both Alaskan specimens. Ushakov (1955) observed that notosetae occur from the second setiger as is the case with the Californian animal discussed below. I consider Annenkova's text on the occurrence of notosetae ambiguous ("Notosetae occur in all dorsal rami of the parapodia," translated from Annenkova, 1934, p. 326) because dorsal rami are not present on all setigers. The diagnosis therefore disregards Annenkova's statement about the notosetae. Moderately numerous, oval eggs in an Alaskan specimen have a size up to about 200 × 250 μ.

The Californian animal, a female with immature eggs, is excellently preserved and has at least 130 setigers; the palps are about as long as the anterior 15–20 segments. Thoracic neuropodial lobes are almost as large as those in *A. frontifilis* (Fig. 4*b*); however, notopodial cirri are absent. The neuropodia are inconspicuous in the Alaskan specimens (almost as in Fig. 5*b* for *A. trisectus*) but I take this to be due to preservation. Also in the otherwise well-preserved specimen of *A. frontifilis* from the Arabian Sea, some feet of exposed segments look like those of the Alaskan animals where the setae did not offer protection against damage after preservation; usually, one can see even then that there had once been some lobes (regretably, there are no figures for the type of *A. heterochaetus*). The abdominal parapodia of all specimens are as figured by Ushakov (1955, fig. 112B).

Posterior notosetae in the Californian specimen are slightly longer than the body is wide. According to the label, the animal that now has a fairly dark, greyish color (as do the Alaskan specimens) was bright orange–yellow in life.

Distribution — Mainland coast of Sea of Japan and to 1500 m depth; Sea of Okhotsk; southwestern Bering Sea; Pacific coast of Alaskan Peninsula; southern California.

Acrocirrus okotensis Imajima

Acrocirrus heterochaetus okotensis Imajima, 1963, p. 189.

Diagnosis — An *Acrocirrus* species with neurosetae from segment 4. Notosetae from segment 6. The first 10 neuropodia with compound hooks. Segment 14 with a heavy acicular hook with a broad head in each parapodium, the following segments with simple bent hooks each with a small spur below the tip.

The species is distinguished from *A. trisectus*, which also has simple hooks in the middle part of the body, by the presence of compound setae on segment 13, by the acicular hook on segment 14, and by the small spur below the tip of the simple hooks. The posterior end is not known; *A. trisectus* has here compound neurosetae.

Material studied — None.

Remarks — The form differs markedly from the nominate subspecies, particularly by the abdominal neurosetae, and is therefore raised to specific rank.

Dr M. Imajima, National Science Museum, Tokyo, kindly communicated to me that the end of the last line on p. 189 of the original description (Imajima, 1963) should read "...the eleventh setigerous segment (fourteenth segment)..." and in the second line on p. 190 "...eleventh setigerous..." He also sent a schematic presentation of the setation and noted that the blades of the hooks on segment 13 face forward, contrary to those of the preceding segments.

Distribution — Northeastern Sea of Okhotsk.

Acrocirrus trisectus n.sp.

Fig. 5

Acrocirrus validus Ehlers 1907, p. 23.

Holotype — BMNH 1907:5:1:41. A well-preserved specimen with about 205 setigers, 12.5 cm length and about 3 mm greatest width. Detached gills are up to 6 mm long and 1 mm thick. A detached palp is slightly longer than the largest gill. Type locality undefined: Portobello, Auckland Harbour, Takapuna. Under stones and in twisted masses in the intertidal.

Diagnosis — A large *Acrocirrus* species with neuropodial hooks from segment 4 (5?). Notosetae from segment 6. Compound hooks in the anterior and posterior part of the body, simple hooks from segment 13 through segment 41.

Acrocirrus trisectus resembles *A. okotensis*, as far as can be judged from the first 27 setigers known in the latter species. However, the new species has compound hooks only through segment 12, lacks a heavy acicular hook on segment 14, and has simple hooks without the spur below the tip present in *A. okotensis*.

The name refers to the three body sections suggested by the change of hooks.

Material studied — Holotype.

Description — The prostomium (Fig. 5*a*) is divided by a transverse ridge into a triangular forward part that protrudes between the scars left by the palps, and a posterior, rhomboid part that carries two eyes and a central ridge as figured. The cushionlike elevations below the palps are low but very broad. Several lines run over the lateral and dorsal sides of the front end behind the prostomium so that it is difficult to decide which represent segmental borders. One interpretation, suggested in Fig. 5*a*, is that $\frac{\text{I}}{\text{II}}$ indicates where the border between the first and the second segment (first gill-bearing segment) is believed to reach the dorsal side. Alternatively, the second segment touches the prostomium so that the first segment reaches only half way up the side of the front end. Large papillae above the gills on the second segment are absent. The large papillae on the third segment are broken off on both sides.

The underside of the body is flat. The parapodia insert at the turn from the lateral to the ventral surface. The fourth segment has a fully developed neuropodium but the setae seem to have broken off. A conspicuous double row of papillae extends between the neuropodium and the gill scar. An irregular single row is found on the next setiger in the same place, and on the following setigers similar rows of papillae are present between the notosetae and the neurosetae. The neurosetae, through segment 12, are surrounded (above and behind the setal sac) by a ridge (Fig. 5*b*), which continues ventrally and bears the papillae common in *Acrocirrus* species in this

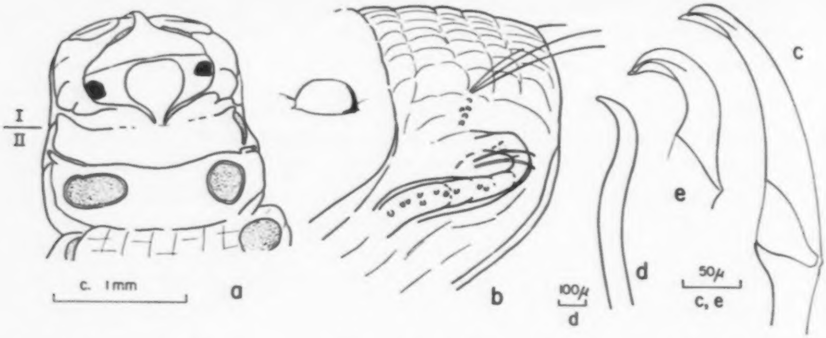

FIG. 5. ***Acrocirrus trisectus*** n.sp.: (*a*) anterior end from above (see text for lettering); all appendages lost; (*b*) seventh parapodium from the left and from below (the insert indicates the part of the segment shown); (*c*) distal part of thoracic compound hook; (*d*) distal part of simple hook from 30th parapodium; (*e*) distal part of compound hook from 110th parapodium.

position. The ridge is reduced on segment 13 and is absent thereafter, although the row of ventral papillae is still present, so that the neuropodia become almost as inconspicuous as the notopodia.

From segment 42, the body is abruptly four-fifths as thick as the foregoing part, and the color and the rugose pattern of the epidermis are slightly changed as if this were a regenerated section. Notopodia and neuropodia are displaced, without transition, somewhat more ventrally than before this segment. On one side above the notopodium of segment 42 a slender cirrus is present. It is similar in shape to a cirratulid gill but transparent and with a central canal, and is quite unlike the gills of Acrocirridae. The cirrus is about 1.5 mm long, and its diameter is slightly less than one-tenth of its length. Since the setal pattern changes at segment 42, I regard the change of body diameter not to be a sign of regeneration.

The neuropodia of segments 5 through 12 have two to three compound hooks (Fig. 5*c*), the cutting edges of their blades facing rearward. There is no heavy acicular hook on the 14th segment but from the 13th through the 41st segments occur two to three simple, thick, and slightly bent hooks of a yellow-brown, somewhat brassy color, which are almost 100 µ thick (Fig. 5*d*). Their distal ends are smooth, without teeth below the tips. They protrude only slightly from the feet, and cutting edges face upward. These hooks appear striated longitudinally throughout, as are the compound hooks on their bases. In addition, there are internal faint transverse lines, 7–10 µ apart. Similar lines occur in the bases of the ventral hooks of some species of Flabelligeridae, i.e., *Brada sachalina* Annenkova. In that species the segmentation at the base is as faint as in *A. trisectus*; the "joints" in *B. sachalina* are not nearly as long as in the middle and the distal part of the setae, and not so distinct either. One to two simple hooks and one to two compound hooks occur on the 42nd segment, which carries a cirrus, and on a few following setigers. Thereafter, only three to four compound hooks with the usual shortened blades (Fig. 5*e*) are present. Notosetae start from segment 6. In the anterior half of the body, two to four notosetae, that have rings of conspicuous spines 2.5–3 µ apart are found per ramus. In the posterior half their number is usually one to two per ramus. The notosetae are about half as long as the body is wide. They become markedly thinner from segment 42.

Very few epidermal papillae are present. The body surface appears rugose dorsally and laterally from the 4th segment (Fig. 5*a*, *b*), and remains so to the tail end, owing to the appearance of usually three to four, occasionally five, pseudoannuli per segment; the pseudoannuli have longitudinal, irregular folds. The color of the animal is dark grey when preserved but dark green in life (Ehlers, 1907).

Distribution — New Zealand.

Macrochaeta Grube, 1851

Type species *Macrochaeta clavicornis* (M. Sars)

Ledon Webster and Benedict, 1887.

Diagnosis — Acrocirridae with short palps, with bases ordinarily separated by at least the width of the palps. Usually four pairs of gills. Beginning of abdomen at a variable segment. Parapodial structures inconspicuous. Epidermis with very numerous, irregularly arranged papillae that are covered with fine sediment particles. Grown specimens of less than 15-mm length and 1-mm width, mostly with fewer than 50 segments. Some species phosphorescent.

The species of *Macrochaeta* are tiny and slender. They are united primarily by their habitus, which is dominated by the epidermal papillae.

Remarks — A profound diagnostic character within the genus ought to be the degree of reduction of the first, and eventually of the second segments (cf. *M. clavicornis* with *M. pege* n.sp. and *M. papillosa*). However, this is not easy to recognize in inadequately preserved material. The number of gills, usually four pairs, must also be considered as an important character for the time being. It is to be checked on new material whether there are really more than four pairs in *M. sexoculata* or less in *M. polyonyx*. The number of eyes is not a useful character, judging from the variability among specimens of *M. clavicornis* from the same sample. Owing to the epidermal papillae, the nephridial papilla below the gill on segment three is often hard to make out, and may perhaps be entirely absent in some species. Likewise, podial interramal papillae are usually difficult to recognize. In *M. clavicornis* where a rather large sample could be studied, the notosetae can appear on the first, second, or third setiger: thus, no emphasis should be placed on this feature in this genus at the present time. The kind of neurosetae (compound hooks only, or others present too), and their number per ramus are characteristic. The length of the blades may be less useful since it usually varies between thorax and abdomen (see, however, Eliason, 1962). The reversal, if present, of the direction in which the cutting edges of the blades of the hooks are facing can also be easily seen unless the specimens are coiled. Little, however, can be made of the epidermal papillae at present: even on the same specimen of *M. clavicornis* the papillae may be 40 or 70 μ long, depending on the contraction. Animals can appear as either shaggy, or rather smooth, in which case the papillae are contracted and the sediment cover of the body is conspicuous.

The arrangement of anterior segments in the one Antarctic species (*M. papillosa*) is rather different from that of the other species of the genus. Also, this species has fewer reduced family characters than do other *Macrochaeta* species.

Ledon is regarded here as a synonym of *Macrochaeta*. Webster and Benedict (1887) established the genus as different from *Macrochaeta* on the basis of a description of *M. clavicornis* from Madeira by Langerhans (1881). That record was depicted as having a very long first segment. Webster and Benedict took the first gill-bearing segment of their animals to be the first segment; however, since the next segment has gills and the pair of cirruslike papillae, and the following segment is the first one with neurosetae, there is every reason to assume that the arrangement of segments in the material of Webster and Benedict is the same as that of *Macrochaeta*.

KEY

1	Many neurosetae per ramus	*M. polyonyx*
	One or two neurosetae per ramus	2
2	Five to six pairs of gills	*M. sexoculata*
	Four pairs of gills	3
3	Some neurosetae pseudocompound or simple	*M. helgolandica*
	All neurosetae compound	4
4	First segment visible dorsally (Fig. 6*a*)	*M. clavicornis*
	First segment not visible dorsally (Fig. 8*d*)	*M. pege*
	First and second segments not visible dorsally (Fig. 7*b*)	*M. papillosa*

Macrochaeta clavicornis (M. Sars)

Fig. 1*f*, 6

Nais clavicornis M. Sars, 1835, p. 64.

Macrochaeta clavicornis Southern, 1914, p. 120; McIntosh, 1923, p. 492 (synonymy); Southward, 1956, p. 272; Eliason, 1962, p. 271; Bellan, 1963, p. 53, 1964, p. 121.

? *Macrochaeta clavicornis* Langerhans, 1881, p. 95; Caullery and Mesnil, 1898, p. 111.

Not *Macrochaeta clavicornis* Fauvel, 1936a, p. 75 (see under *Acrocirrus frontifilis*).

Not *Macrochaeta clavicornis* ? Banse et al., 1968, p. 541 (see under *M. pege* n.sp.).

Types — Neither the types nor other *Macrochaeta* material of Sars are in the Zoological Museum in Bergen, Norway (Prof H. Brattström, University of Bergen, personal communication) nor at the University of Oslo. It is most likely that they had been deposited in Bergen if material had been kept at all (Mr C. Støp-Bowitz, University of Oslo, personal communication). The type locality is mud (gyttja) bottom near the city of Florö, near Bergen, at approximately 61°40′N, 5°00′E.

Diagnosis — A *Macrochaeta* species of up to 1 cm length and at least 45 segments. First segment visible dorsally. Four pairs of gills. One to two notosetae and compound neurosetae per parapodium. Reversal of hooks usually from segment 13. Large epidermal papillae.

Macrochaeta clavicornis is distinguished from the two other species that have four pairs of gills and a few neurosetae that are all compound (*M. papillosa* and *M. pege* n.sp.) by the dorsally visible first segment.

Material studied — Elly Bay, Blacksod Bay, northwestern Ireland. From surface tow net, station W. 115 (for locality see Southern, 1915, pl. I), September 13, 1909. NMI No. 283, 1909, one specimen. The full collection from the Clare Island Survey treated by Southern (1914) is at neither NMI nor BMNH. One specimen of the collection, now at neither the Gatty Laboratory nor BMNH, was the basis for the description by McIntosh (1923).

Blacksod Bay, northwestern Ireland, 7 m (4 fath; from label). Collected and identified by Irish Fisheries, BMNH No. 1914:12:12:40, four specimens. These specimens do not seem to be recorded so far. Southern had found the species in the same location earlier.

Irish Sea, stations 20, 30, 33, and 66, of Southward (1957). Dr E. C. Southward's collection (Southward, 1956), six specimens.

Kattegat, East Lille Middle Ground, 56°57′N, 12°06.5′E, sand, gravel, stones, *Laminaria*, 21 m, July 5, 1937, five specimens. Collected by L. A. Jägerskiöld; identified by Dr A. Eliason (Eliason, 1962). "Skagerrak" station 142; NMG, Polych. No. 6517. The station is now numbered as Kattegat station No. 337 in the catalog of the NMG (from the label, and Dr A. Eliason, Naturhistoriska Museet, Göteborg, personal communication).

Mediterranean Sea, Archipel de Riou, near Marseille, coarse sand with small pebbles ("*Amphioxus* sand"), 18 m; incorporated in Bellan (MS, 1964). SME, about 15 specimens.

New record: North Sea, near the northern islet of Kleppholmen, 60°11.3′N, 05°08.7′E, near Bergen, Norway. Shell sand, 25–30 m, October 22, 1962. Station 338-62, collected and identified by Dr A. Eliason. Dr Eliason's collection, two specimens.

New record: Skagerrak, at Gunnarskär, 58°17.7′N, 11°16.3′E, western Sweden, shell sand, 20 m, May 13, 1965. Collected and identified by Dr A. Eliason. USNM 39319, eight specimens. BMNH No. 1968:138, two specimens.

Redescription — The redescription is based on the material from Gunnarskär. The 10 well-preserved specimens have a maximum of 35 setigers and are up to 6–7 mm long. A relaxed, almost colorless anterior fragment of 21 segments, 5 mm long and up to 0.3 mm wide is shown in Fig. 6*a*.

Anteriorly is a pair of frontal palps. On the prostomium are three pairs of eyes, the smaller ones being deep below the skin, and scattered brown pigment spots. In specimens from other localities the smaller eyespots may be missing. The prostomium lies dorsally on the papillated first segment (Fig. 6*a*, *b*). The latter is clearly visible dorsally, as well as laterally, where it is completely differentiated from the second segment. Ventrally, an extension of the first segment reaches to the third segment (Fig. 6*c*). The second to fifth segments each carry a pair of gills in various states of regeneration. The gills are free of epidermal papillae, as are the palps. There is a large hollow papilla on the dorso-lateral anterior margin of the third segment, below the gill.

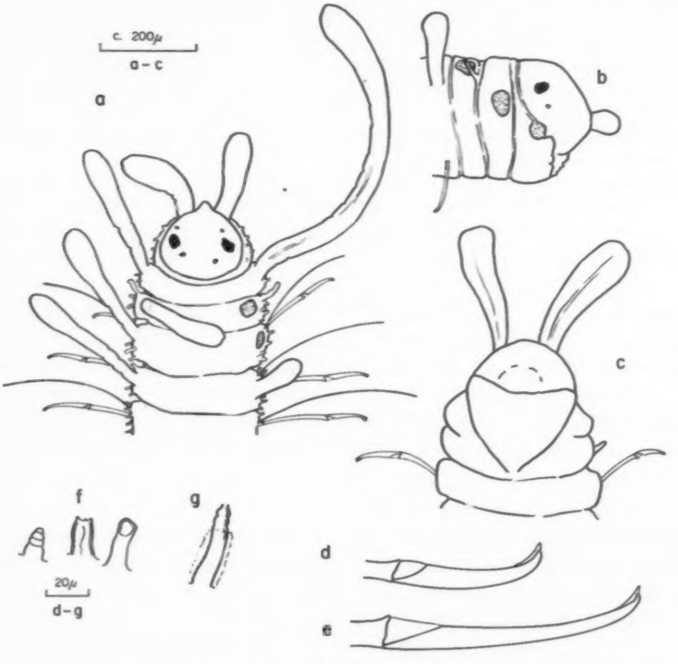

FIG. 6. *Macrochaeta clavicornis* (*a*, *c*, from Skagerrak; *b*, *d–f* from North Sea; *g* from Ireland): (*a*, *b*, *c*) dorsal, right side, and ventral view of anterior ends; epidermal papillae indicated in *a*; (*d*) thoracic hook; (*e*) abdominal hook; (*f*, *g*) epidermal papillae.

Anteriorly, the parapodia insert in the forward parts of the setigers, posteriorly, in the last third. Particularly in the posterior portions of the animals, three to four podial papillae, apparently interramal and about 50 μ long, stand out clearly from the other lower epidermal papillae. They are of the same form as the latter, i.e., secretory. Anteriorly, there is usually one capillary and one compound seta per parapodium; after the reversal of the hooks, two hooks, and often two capillary setae are usually found per ramus.

Compound hooks (Fig. 1*f*, 6*d*, *e*) start on the fourth segment in all animals. The blades of hooks occurring prior to the segment of reversal tend to be somewhat longer than those of the posterior portion of the body (Fig. 6*d*, showing an extreme case, and Fig. 6*e*). In all nine specimens from Gunnarskär in which the hooks could be clearly made out, reversal of the direction in which the blade is facing occurs from the 13th segment. In eight of these specimens, which had 25–35 segments, the regularity of forward-facing blades was checked on one side of the body: In six animals, there was no exception; in one, there was one hook reversed in a posterior segment, and in the eighth specimen, partial damage did not allow precise observation. Needlelike aciculae are found in both rami.

Notosetae start on the fourth segment (first setiger) in eight of nine specimens, and on the fifth segment in the ninth one. These setae are very slightly spinose, and up to 0.6 mm long. Swimming setae are absent in the material from Gunnarskär, but start on the 10th segment in an anterior fragment of a specimen from Bergen, and in two specimens from Blacksod Bay (NMI; BMNH). In the latter animals, the swimming setae are 0.9–1.0 mm long whereas the ordinary capillary setae are 0.5–0.6 mm long; about half a dozen setae per ramus occur on at least 11 (possibly 13) segments.

Most epidermal papillae of the animals from Gunnarskär are of uniformly low height (15–20 μ) and almost covered with detritus; occasionally there are papillae up to 50 μ long. The figures show papillae drawn from animals from different localities (Fig. 6*f*, *g*). The distance between papillae apparently varies with the contraction of the animals but ordinarily is 20–40 μ.

In many specimens, although not in those from Gunnarskär, the prostomium and the first segments are dusky colored. The pigment is particularly concentrated in the segmental borders and makes the borders appear clearly.

The greatest length reported for the species is 1 cm (McIntosh, 1923). I have seen many animals of 7 mm length. The number of segments is usually below 40, but in the sample from the British Museum listed above, a 5-mm long incomplete animal has slightly more than 40 setigers, i.e., at least 45 segments.

Remarks — In two specimens from northwestern Ireland (BMNH 1914: 12:12:40), it could be recognized that the blades of the hooks face rearward through segment 11 (one specimen) or 12 (one specimen). In three specimens where this could be made out, the blades of the hooks face consistently forward after the segment of reversal. The first segment is dorsally visible in these specimens (as well as in most of Southward's material from the Irish Sea). Thus the Irish material agrees very well with the Norwegian.

The moderately well-preserved animals recorded by Southward (1956) from the Irish Sea are distinguished by the fact that the blades of many posterior hooks face rearward, apparently in an irregular pattern. An individual from sample 30, from coarse gravel and muddy sand, had bolusses of very fine material in the intestine. Another one in sample 20, from shell and sand gravel, had intestinal contents of particles of $\leq$20-μ size, indicating selective feeding on quite fine materials.

In material from the Kattegat, reported upon by Eliason (1962), the long papilla on the third segment is almost 100 μ long, an extraordinary length for the species. Podial papillae of two of the five poorly preserved specimens are conspicuous, being 75 μ long. In the two specimens where this could be studied, irregularities again seem to occur in the direction in which the blades of the posterior compound hooks are facing. One animal carries fairly numerous round eggs of about 40-μ diam from the 13th segment. Swimming setae are not present.

The material from Marseille consists of anterior ends only. The first segments of many specimens are dorsally half as long as each of the next segments, a condition approaching that of material from Madeira (Langerhans, 1881). Two interramal papillae per foot, 30–50 μ long and with globular tips, are conspicuous.

Distribution — North Sea, into the Kattegat, and around the British Isles, western Mediterranean (first recorded here by Pérès, 1954; the material seems to be lost). A further record comes from Roscoff in northwestern France (Cabioch et al., 1968) for which the material is no longer available (Mr J.-P. L'Hardy, personal communication).

The record from Madeira of mature females (Langerhans, 1881) is distinguished by a very large first segment and warrants new investigations. Caullery and Mesnil (1898) saw one of Langerhans' specimens and found six to seven swimming setae per ramus from the eighth setiger; in the north European form of *M. clavicornis*, they occur from the seventh setiger. This material cannot be found in the Zoological Museum of the University of Freiburg (Prof K. Sander, University of Freiburg, personal communication) nor the ZMB (Dr G. Hartwich, Humboldt-University, Berlin, personal communication; Langerhans' travel had been supported by the Academy of Sciences, Berlin, the collection of which are in the mentioned museum), nor the Zoological Museum of the University of Vienna (Dr E. Pfiffl, University of Vienna, personal communication; Caullery and Mesnil received one of Langerhans' specimens through von Marenzeller in Vienna).

Macrochaeta sexoculàta (Webster and Benedict)

Ledon sexoculata Webster and Benedict, 1887, p. 744.

Type — No material of *Ledon* is in the catalogued collection of the USNM where Webster's types have been kept (Dr M. H. Pettibone, Smithsonian Institution, Washington, D.C., personal communication).

Diagnosis — A *Macrochaeta* species 6–8 mm long. First segment dorsally not visible (?). Five to six pairs of gills. One notoseta and one to two neurosetae per parapodium. Anterior part of body covered densely by epidermal papillae.

The chief distinguishing characters of this species are the five to six pairs of gills on as many segments. Presumably, all neurosetae are compound.

Material studied — None.

Discussion — The synonymy of *Ledon* Webster and Benedict with *Macrochaeta* has been discussed on p. 2609. The unusual character of five or six pairs of gills makes the finding of new specimens very desirable.

Distribution — New England.

Macrochaeta papillosa Ehlers

Macrochaeta papillosa Ehlers, 1913, p. 539; Fauvel, 1936b, p. 31; Hartman, 1966, p. 31.

Types — Lectotype: ZMB No. 5894a (designated here), Winterstation, Deutsche Südpolar Expedition, off Wilhelm II Land, 66°02′S, 89°38′E, 385 m, November 22–24, 1902 (phosphorescent, from label). An anterior fragment of 24 segments, about 8 mm long.

Paralectotypes: (about 20), ZMB No. 5894b (designated here). For locality see above; 380–385 m, 1902–03 (former numbers of collection 5894 and 6761).

Diagnosis — A *Macrochaeta* species of up to 1.1 cm length, and about 50 setigers. Insertion of palps close together. Pillowlike elevations below the palps. First and second segments dorsally not visible. Four pairs of gills. Conspicuous interramal papillae. Two to three notosetae and one to two compound neurosetae each per parapod. Reversal of hooks from segment 12 or 13. Large epidermal papillae usually present.

The distinguishing features of this species are the dorsal reduction of the first and second segments, the obvious presence of pillowlike elevations below the palps, and large podial papillae. The insertion of the palps resembles that of *Acrocirrus* species but the habitus (conspicuous epidermal papillae, few neurosetae) and the variable length of the thorax suggest an inclusion of the species in the genus *Macrochaeta*.

Material studied — Type series (see above).

Winterstation, Deutsche Südpolar Expedition (for locality see above), December 6, 1902, 385 m. HZM No. V.8595, two specimens.

Alexander Island, Bellingshausen Sea (recorded by Fauvel, 1936b). ISNB, two specimens.

Additions to the description — The entire material of Ehlers has been available. Figure 7*a* depicts the same specimen as fig. 16, pl. 41 by Ehlers (1913). In the few animals where the proboscis is not extruded, the prostomium occupies the upper half of the rather bluntly truncated front end. In the other specimens, it is pushed upward (Fig. 7*b*). Pillowlike elevations below the insertion of the palps, known from *Acrocirrus* spp., are well marked. The first segment is dorsally reduced as is the second segment; both are dorsally not visible (Fig. 7*a*, *b*). As noted earlier, the species is outstanding in this regard among the Acrocirridae. The fourth segment projects dorsally between the gills on the third segment in the specimen drawn in Fig. 7*a*, but usually the anterior margin of the fourth segment is almost straight. Ventrally the second and third segments are fused but usually clearly separated from the first segment because of the different color of the latter. There are four pairs of gills (clearly seen as such, or by their scars, only on five out of the 22 specimens).

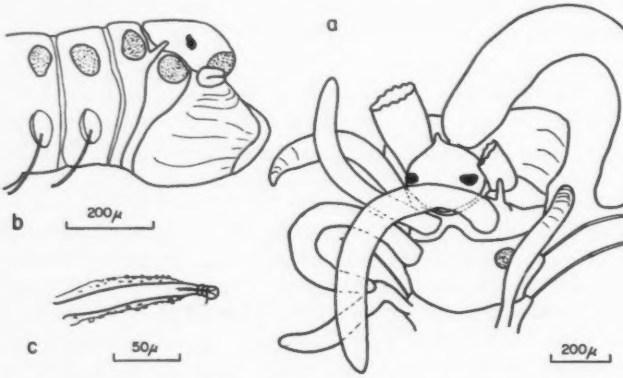

FIG. 7. *Macrochaeta papillosa*: (*a*) dorsal view of anterior end of specimen collected on April 7, 1903 (ZMB No. 6761), epidermal papillae omitted; proboscis everted; (*b*) sketch of anterior end of lectotype, from the right side, proboscis everted; (*c*) long epidermal papillae, with detrital material indicated.

The neuropodia are easily visible in specimens with smooth epidermes as oval elevations, as are long interramal papillae. Compound hooks of the usual form start on the fourth segment; capillary setae start on the sixth. The cutting edges of the blades of the hooks face rearward until the 11th or 12th segments (in four specimens each), and face consistently forward thereafter. Usually one hook per ramus is present in the thorax and two in the abdomen. Two to three notosetae are found in all parapodia. Of the 22 specimens collected between November 1902 and July 1903 on the winter station of the German South Polar Expedition, three have swimming setae (collected in November and December, among several specimens that do not have them). About a dozen of these setae per ramus, up to 2.5 mm long, occur from the eighth or ninth segment; as is usual in the genus, they are smooth proximally and segmented and spinose distally. Polygonal eggs in a specimen (HZM) without swimming setae have a diameter of about 150 μ.

Epidermal papillae are unusually long in many animals (Fig. 7*c*), making these specimens appear shaggy. Some papillae, on the second segment near the gill scar, are directed forward, like most interramal papillae, and look similar to the large papilla on the third segment (see Fig. 7*a*, *b*, and *A. heterochaetus*, which has a large papilla on the second segment). As already stated by Ehlers, other specimens are almost smooth; it is remarkable that these have almost no sediment on the skin. Both appearances can occur in the same samples.

The "Belgica" material (Fauvel, 1936b) had once been dried up. The still recognizable features (size, setation, and parapodial and epidermal papillae) are the same as those of Ehlers' species.

Distribution — Antarctic, in Indian and Pacific Ocean sectors (not Ross Sea, as in Hartman, 1966).

Macrochaeta helgolandica Friedrich

Fig. 8*a–c*

Macrochaeta helgolandica Friedrich 1937, p. 347.

Types — The types no longer exist (Prof. H. Friedrich, Überseemuseum, Bremen, personal communication). The type locality is the "*Amphioxus* ground" near the island of Heligoland. This ground, where dredgings have been made for decades by the Biological Station at Heligoland, is approximately a rectangle with the southwestern corner at 54°15.3′N, 07°55.9′E, and extending 0.4′ of latitude to the North, and 1.2′ of longitude to the East (Dr W. Gunkel, Biological Station, Heligoland, personal communication). The depth is 19–21 m, with coarse sand.

Diagnosis — A small *Macrochaeta* species of about 0.5 cm length. Peristomium dorsally not visible. One to two notosetae and neurosetae each per parapodium. Some neurosetae simple or pseudocompound, with lamellae at the tip. Hooks face irregularly forward or rearward. Epidermal papillae small.

The principal distinguishing character of this species is the occurrence of pseudocompound or simple hooks with the common lamellae at the tips. These are not known from any other species of the family.

Material studied — "*Amphioxus* ground" near Heligoland, August 1963 and 1964. Collected and identified by Dr W. Westheide from two sites. USNM No. 39165, three specimens.

New record: At Bonden, western Sweden, about 58°12.7′N, 11°19.1′E, shell sand, 25 m, May 29, 1964. Collected and identified by Dr A. Eliason. Dr Eliason's private collection, two specimens.

Addition to the description — In five specimens from Heligoland (up to 3.3 mm long and 0.12 mm wide, with up to 20 setigers, incomplete) the first segment is dorsally not visible but is laterally clearly separated from the second segment. The third segment is the first setiger. Notosetae occur from the fifth segment. Parapodia are in the rear part of the segments from the sixth segment. Contrary to the two type specimens, often only one capillary seta and one hook may be found in a parapodium past the eighth segment as in the first setigers. Between the 7th and the 16th segments, frequently hooks are found that are not truly compound (as in Friedrich, 1937, fig. 8*a*), or are not compound at all; these hooks have lamellae at the tips of the blades (Fig. 8*a*). After the first few setigers, no pattern is apparent of the direction in which the cutting edges of the blades of the hooks face. Only scattered epidermal papillae, 4–6 μ high and 3 μ wide (Fig. 8*b*) are present, but much detritus sticks to the skin. Owing to the smallness of the epidermal papillae, podial papillae between the rami and a ring of about 10 papillae on the pygidium (Fig. 8*c*) stand out (the latter seem to be present also in *M. clavicornis*).

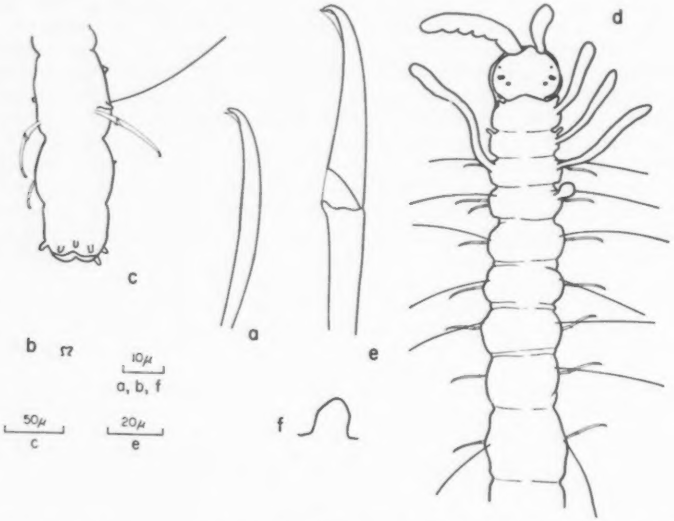

Fig. 8. *Macrochaeta helgolandica* (from Heligoland): (*a*) simple hook; (*b*) epidermal papilla; (*c*) ventral view of posterior end; epidermal papillae indicated on the sides; ***Macrochaeta pege*** n.sp.: (*d*) dorsal view of anterior end; epidermal papillae omitted; (*e*) thoracic hook; (*f*) epidermal papilla.

The specimens from Sweden, largely identified by their setae, agree.

Distribution — Southern North Sea; Skagerrak.

Macrochaeta polyonyx Eliason

Macrochaeta polyonyx Eliason, 1962, p. 269.

Types — Holotype: Zoological Institute, University of Uppsala, Sweden; 58°30′N, 10°26′E, Skagerrak, from grey mud, 300 m. Paratypes (15 specimens): in the same collection.

Diagnosis — A *Macrochaeta* species of at least 12 mm length and at least 36 segments. First segment not usually visible dorsally. Two pairs of gills. At least anteriorly, up to 10 compound neurosetae with slim blades per parapodium, at least some hooks reversed posteriorly. Blades of hooks longer posteriorly than anteriorly. Rather large epidermal papillae.

The species is distinguished from the other *Macrochaeta* species by the numerous neurosetae in the anterior segments. The fact that the blades of the neurosetae become larger posteriorly rather than shorter is noteworthy.

Material studied — New record: Raunefjord, near Bergen, Norway, very approximately 60°16′N, 5°09′E, sand-mixed clay, 245 m, October 5, 1962. Collected and identified by Dr A. Eliason. Dr Eliason's private collection, four specimens.

Remarks — The moderately well-preserved material from Norway agrees with the description by Eliason (1962). The first segment cannot be recognized from the dorsal side. Segments 2 and 3 are short, and only pigment indicates what may be the dividing line; they are separated from the prostomium and segment 4 by deep furrows. The papilla below the second gill is very conspicuous. No gill scars can be seen on the following segments, even on the two pigmented specimens. Eliason also had not seen any gills on these segments in his 71 specimens, which, however, also lacked palps.

Notosetae are present from segment 4, the first setiger. In posterior segments (at about segment 20), only one to three hooks may occur, or four to five (as described by Eliason, 1962). As also observed by me, blades become longer posteriorly. All blades face rearward in the first dozen or so setigers; thereafter at least many if not most face forward.

Distribution — Skagerrak, northern North Sea.

Macrochaeta pege n.sp.

Fig. 8*d–f*

Macrochaeta clavicornis? Banse et al., 1968, p. 541.

Types — Holotype: USNM No. 39163, from coarse sediment out of one of two dredge hauls, the first one on sand from about 60 m between Reid Rock and Turn Island, near San Juan Island, Washington (approx 48°32.5′N, 122°58.5′W) in summer 1961, the second one at a nearby station in rather clean sediment mostly made up of broken shells, from about 100 m, in summer 1962. Paratypes (eight specimens): from the same localities, USNM No. 39164.

Diagnosis — A *Macrochaeta* species of up to 7 mm length and 55 segments. First segment dorsally not visible. Four pairs of gills. One or two notosetae and compound hooks each per parapodium. Reversal of hooks from segment 13. Small epidermal papillae.

This species, the only one known from the Pacific Ocean, has the largest number of segments among the members of the genus. It is distinguished from the two other species that have four pairs of gills and a few neurosetae that are all compound (*M. clavicornis* and *M. papillosa*), by a first segment that is dorsally not visible.

The name is derived from πηγή to indicate that this study sprang from the finding of this species.

Material studied — Type series (see above) and about 10 more specimens from the same localities. About 10 other, poorly preserved fragments come from station 5 of Lie (1968), near Seattle, May 1963 (USNM No. 39282). Figure 8*d* is drawn from a living specimen, which is not among the types, from near San Juan Island.

Description — The largest anterior fragment with 20 setigers is 5 mm long and 0.4 mm thick. A complete specimen has 55 setigers, and is almost 7 mm long and 0.2 mm thick. The other fragments are about half as long, and vary in thickness between the two measures given.

The prostomium has a frontal projection between the origin of the palps (Fig. 8*d*). In preserved material from 1963, the posterior margin appears to be rounded such that the prostomium is almost circular. Two large brown or black eyes are accompanied by two small pigment spots deep below the skin. The latter spots are most often behind the large eyes but occasionally they occur in front of them. Ciliated nuchal grooves are present on the lateral-posterior margin, and are visible also in the mentioned preserved specimens of 1963. The first segment, not discernible from above in living or preserved animals, is laterally clearly visible in living material. The border to the second segment is difficult to recognize — if it can be seen at all — in preserved specimens. The first segment seems to be devoid of epidermal papillae.

The species has four pairs of gills that are usually lost in dead animals; below the second pair, a cirrus can sometimes be seen. The fourth segment is the first setigerous one and has noto- and neurosetae. The setae of the first seven setigers arise in the anterior third, and subsequently in the posterior half of the segments. Parapodia as such are not visible, but usually a small podial papilla can be distinguished. One notopodial spinose capillary seta, one and one-half to two times as long as the body is wide (Fig. 1*e*), is accompanied by three to four needlelike aciculae, which are as thin as the setae and do not protrude. The neuropodia contain one, or occasionally two, aciculae of the same form as the notopodia, and one or occasionally two compound hooks (Fig. 8*e*). The cutting edges of the blades of the hooks face rearward in segments 4–12; thereafter, they face consistently forward. From about that point, the segments become twice as long as they are wide. The segments are again short at the posterior end. The pygidium is blunt, and without appendages in the preserved material, but there are papillae of approximately the same form as in *M. helgolandica* (Fig. 8*c*).

The body is adhesive. Papillae of 10–20 μ length (Fig. 8*f*) are irregularly arranged. In animals of normal contraction, the papillae are about the same distance from each other. The color of small specimens is whitish, that of larger ones is brownish, particularly so on the front end, owing to many chromatophores in the epidermis.

Distribution — Puget Sound, Washington, and adjoining waters.

SPECIES INQUIRENDAE

Macrochaeta leidyi (Verrill)

Acrocirrus leidyi Verrill, 1882, p. 370 (footnote); Hartman, 1944, p. 334, 341 (Verrill's figures are provided here).

? *Acrocirrus leidyi* Hartman, 1942, p. 66.

Ledon leidyi Hartman, 1959, p. 409.

Type — PMNH 24-10 (in 1967, when seen by me, three pieces, without anterior or posterior ends, together making up about 20 setigers).

Remarks — This is a species from New England of uncertain standing as already noted by Hartman (1942). It certainly belongs to *Macrochaeta* as defined here, on account of the habitus, the epidermal papillae, and the setation. The species is the largest one of the genus, being up to 15 mm long, but it cannot be characterized because of the state of the type, and because of internal inconsistencies of Verrill's text and figures. The species appears to have four pairs of gills. Regarding Verrill's fig. 5 (on Hartman, 1944, pl. 59) it seems likely — judging from the position of the mouth and the setation — that the gills were inadvertently drawn on the wrong segments; it is less likely that there is a long first segment as in *M. clavicornis* as described by Langerhans (1881). All setigers of the type material have notosetae that are almost twice as long as the body is wide. The blades of the hooks face in the same direction.

Macrochaeta sp.

Macrochaeta sp. Eliason, 1951, p. 134.

The very small fragments were identified by Eliason from the setae only. This is an important record from the western tropical North Atlantic Ocean that extends the distribution of the family to the deep sea (5000 m).

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Glycolytic and Nucleotide Changes in the Critical Freezing Zone, –0.8 to –5 C, in Prerigor Cod Muscle Frozen at Various Rates

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Glycolytic and nucleotide changes during freezing at different rates were investigated in prerigor muscle from unexercised cod. Significant changes occurred during freezing as samples 1 cm thick in the center of a 2.8-cm thick pack cooled through the critical temperature zone from –0.8 to –5 C, but little further change occurred after the temperature had fallen below –5 C. The amount of change depended on the thermal arrest time (TAT) (time from –0.8 to –5 C). During rapid freezing with a TAT of 0.22 hr, the most significant change was a 39% dephosphorylation of creatine phosphate; small amounts of glycogen and acid-labile phosphorus were lost. Freezing at an intermediate rate (TAT, 5.4 hr) resulted in a loss of one-half of the initial glycogen and almost all of the creatine phosphate; also about one-third of the initial ATP was converted to IMP. After slow freezing (TAT, 20 hr) only traces of glycogen and high energy phosphorus compounds remained in most samples.

Dephosphorylation of creatine phosphate, and degradation of ATP to IMP occurred more rapidly in freezing muscle at –1 C than in unfrozen muscle at 0 C. The rates of glycolysis were similar, but when the temperature dropped below –1 C there was an apparent increase in glycolytic activity.

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INTRODUCTION

RECENTLY, interest in freezing fish in prerigor condition has increased with the development of large freezer-trawlers. This method of processing can yield a product that is superior to one produced from postrigor material (Anon., 1968; Peters et al., 1968) but problems associated with the development of rigor are encountered when freezing very fresh fish (see Jones, 1965). Postmortem glycolysis and changes in nucleotides leading to the development of rigor have been studied extensively in iced cod muscle but little attention has been given to changes that occur in prerigor muscle during the freezing process.

As prerigor fish muscle is cooled to 0 C the rates of glycolysis and ATP[1] breakdown decrease (Fraser et al., 1961), but further lowering of the temperature results in an increase in activity followed by a decline to very slow rate

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[1] The following abbreviations are used: ATP, adenosine triphosphate; ADP, adenosine diphosphate; AMP, adenosine monophosphate; IMP, inosine monophosphate; IP, inorganic phosphorus; Δ7P, acid-labile phosphorus; TP, total phosphorus; AN, adenosine compounds; TN, total adenosine and inosine compounds; CP, creatine phosphate; TAT(s), thermal arrest time(s); and TLC, thin-layer chromatography.

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below −5 C (Sharp, 1935; Partmann, 1963). Within the range of −1 to −5 C, enzymic activity is maximal in various marine species (see Love and Elerian, 1964), and also in this range most of the water content is frozen out as pure ice (Dyer, 1968). Therefore, this temperature interval has been designated as the critical freezing zone, and the time required for the center of the sample to pass through it, the TAT.

There is little information in the literature concerning the extent of glycolysis in prerigor cod muscle at different TATs, and the pattern of change as the sample passes through the critical freezing zone has not been defined. Most results from earlier studies represent the net chemical change in muscle after an arbitrary storage period in a constant temperature freezing chamber with neither the actual temperature change of the muscle nor the TAT being determined.

Our study was designed to elucidate the relationship between the postmortem biochemical reactions occurring in cod muscle during freezing and the time taken to pass through the critical temperature range, defining this zone with the use of thermocouples. Specifically, (1) the extent of glycolysis and related reactions was determined at different freezing rates (i.e., at different TATs) spanning the range found in commercial practice, and (2) the pattern of glycogenolysis and of nucleotide catabolism during slow freezing in the critical zone from −0.8 to −5 C was determined and compared to that in unfrozen muscle at 0 C.

EXPERIMENTAL

For the purpose of this study it was necessary to freeze samples in such a way that cooling was as uniform as possible throughout the sample of muscle used. During freezing of a block of muscle the temperature decreases rapidly in the surface layers but the change is more gradual in the central mass (Long, 1955; MacCallum et al., 1965). In the central portion the rate of heat removal is slow enough that the temperature gradient is small, thus providing a portion of suitable size for determining biochemical changes and in which the temperature changes can be determined by thermocouples.

To simplify sampling of this central portion, muscle slices 1 cm thick were sandwiched between two insulating layers of cod fillet 0.8 cm thick, and thin pieces of polyethylene film (1.15 mil) were placed between the samples and the insulating fillet pieces to facilitate separation after freezing (Fig. 1). The assembled samples were placed in a polyethylene bag in a waxed carton measuring 7.8 × 21.6 × 2.8 cm, and scraps of fish were added to fill the box completely.

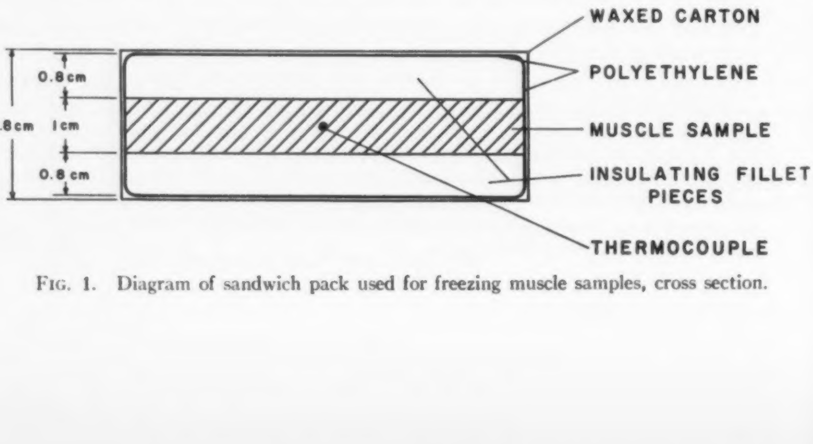


FIG. 1. Diagram of sandwich pack used for freezing muscle samples, cross section.

This package was equivalent in thickness (2.8 cm) to the commercially used 1-lb (454-g) box. Samples for slow freezing were insulated further with two extra layers of waxed carton and several sheets of newspaper around the outside of the box to prolong the TAT and make it comparable to that in the centre of a thick muscle block. Prior to freezing, a copper–constantan thermocouple was inserted into a muscle slice close to the geometric centre of each box, and temperature changes during freezing were followed with a Honeywell-Brown recording potentiometer printing at 1.5-min intervals. Samples were frozen at three different rates: rapidly in a plate freezer, plate temperature at –40 C; at an intermediate rate in a –17 C room; slowly in –10 C air with extra insulation as described above. This resulted in a comprehensive range of TATs.

Samples used in these experiments were from feeding cod (*Gadus morhua*), averaging about 73 cm long, which were removed from the laboratory aquarium with little struggling, stunned immediately by a blow on the head, and filleted at once. The fillets were placed in polyethylene bags in an ice bath and taken to a cold laboratory (3 C) where a number of transverse slices 1 cm thick were cut from the mid to posterior section of each fillet. Red muscle was removed and discarded. Samples for freezing were quickly packed sandwich-style and placed at the appropriate temperature within 40 min post mortem.

Acid-soluble metabolites were extracted from fresh and frozen muscle with ice-cold 0.3 N perchloric acid as described previously (Nowlan et al., 1968). The temperature of frozen muscle samples was not allowed to rise prior to extraction. Unless otherwise indicated, each sample for extraction was obtained from four slices of muscle from one fish by cutting a 5-g portion from the center of each slice and combining these to form a composite sample.

Glycogen was determined as the sum of the acid-extractable fraction and residual glycogen obtained by digesting the tissue residue in 30% KOH (Russell and Bloom, 1955). Aliquots of the neutralized perchloric acid extract were used for determination of lactate, IP, and Δ7P as described previously (Nowlan et al., 1968). Δ7P in this report refers to high energy phosphate derived from ATP, ADP, and CP. CP was determined by the method of Ennor and Rosenburg (1952) and also as the increase in IP after hydrolysis in 0.1 N HCl at 100 C for 1 min. Results from the two methods were comparable in most cases. TP was determined by the procedure of Harris and Popat (1954). TN and AN were calculated from the absorption spectra at 251 mμ and 260 mμ, respectively, as described by Dyer et al. (1966). ATP, ADP, and IMP were separated by TLC followed by elution of appropriate spots and spectrophotometric measurement (Fraser et al., 1967). Samples for *p*H determination were homogenized with 10 volumes of .005 N sodium iodoacetate (Marsh, 1952).

RESULTS

GLYCOLYTIC AND NUCLEOTIDE CHANGES AT DIFFERENT TATs

Freezing curves for samples of prerigor muscle frozen at rapid, intermediate, and slow rates are shown in Fig. 2. Each curve consists of three parts: a fairly rapid period of initial cooling to –0.8 C, a zone of very slow temperature decrease between –0.8 and –5 C designated as the critical freezing zone, and a final zone of rapid cooling to the ambient temperature. The average time in the critical zone for plate-frozen muscle was 13 min and for samples frozen at intermediate and slow rates, the TAT was 5.4 and 19.9 hr, respectively (mean of seven trials at each rate).

In seven fish with varying initial glycogen levels, the pattern of change in metabolite concentration during freezing at the three different rates was comparable (Table 1) and results are therefore summarized in Table 2. At the rapid freezing rate (TAT, 13 min) the most significant change was a 39% average decrease in CP content; also 15% of the initial glycogen and 11% of the Δ7P (excluding CP) were lost. The intermediate freezing rate (TAT,

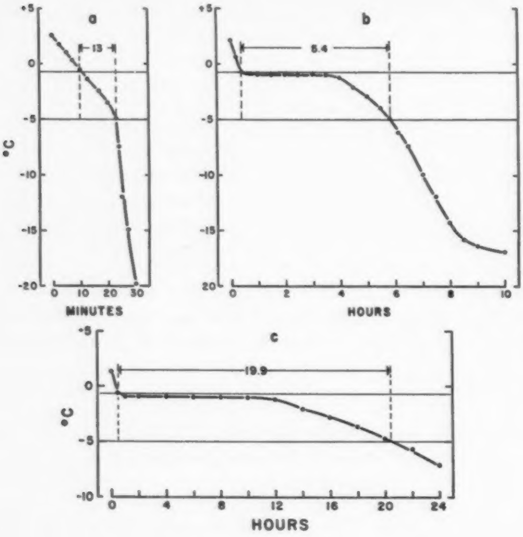


FIG. 2. Freezing curves for prerigor cod muscle frozen: (*a*) rapidly in a plate freezer, plate temperature -40 C; (*b*) at an intermediate rate in a chamber at -17 C; or (c) slowly at -10 C. Slices of muscle 1 cm thick were frozen in the middle of a sandwich pack 2.8 cm thick (Fig. 1) and a thermocouple near the geometric centre monitored the temperature of the muscle. Each point represents an average of seven fish. The critical temperature zones from -0.8 to -5 C are marked and TATs are indicated.

5.4 hr) resulted in a 50% decrease in glycogen content; CP was almost depleted and 29% of the Δ7P was lost. After very slow freezing (TAT, 20 hr) only traces of glycogen and Δ7P remained in most samples, and CP was completely dephosphorylated. In most cases the amount of glycogen lost during freezing was comparable to the quantity of lactic acid formed. The average *p*H decrease corresponded to that expected from the increase in lactic acid (Fraser et al., 1967) in the slowly frozen muscle. The concentration of AN decreased during freezing at both slow and intermediate rates indicating that deamination to inosine nucleotide compounds had occurred.

Thin-layer chromatographic analysis of an extract from one fish (Table 1, fish 7) revealed that rapid freezing did not affect levels of the individual nucleotides, ATP, ADP, and IMP (Fig. 3). At the intermediate freezing rate about one-third of the initial ATP was converted to IMP (increasing the IMP concentration from the initial level of 0.45 to 1.8 mμmoles/g) whereas during slow freezing almost all of the ATP was converted to IMP; in both cases traces of inosine were formed as well but no hypoxanthine was detected. ADP concentration changed very little on freezing and no AMP could be detected in either fresh or frozen samples.

The present data confirm that extensive glycolysis and nucleotide degradation can occur during freezing and that the extent of these changes is related to the TAT (Fig. 4). They are negligible at 13 min, practically complete in 20 hr, and quite extensive at 5 hr in the critical zone. During freezing, CP is depleted most rapidly followed by glycogen breakdown and ATP dephosphorylation and deamination to IMP; however, further dephosphorylation to inosine is minimal.

TABLE 1. Glycolytic metabolite and nucleotide concentrations in prerigor cod muscle before and after freezing at different rates.[a]

Fish	Freezing rate	TAT[b]	*mg/100 g*		Δ7P less CP[c]	*μmole/g*					*p*H
		(*hr*)	Glycogen	Lactate	(*μg-at P/g*)	CP	IP	TP	TN	AN	
1	Initial unfrozen	–	242	75	6.0	10.8	26.7	55.1	6.88	5.96	–
	Rapid	0.13	203	113	5.4	8.7	31.2	54.1	7.26	5.89	–
	Intermediate	5.31	102	178	8.2	0	33.0	54.2	6.22	4.06	–
	Slow	22.2	28	186	0	0	38.1	55.7	6.64	1.96	–
2	Initial unfrozen	–	155	104	10.7	8.7	36.8	61.5	7.96	6.68	7.35
	Rapid	0.17	136	152	9.3	5.3	39.5	59.8	7.69	5.74	7.30
	Intermediate	6.15	84	215	7.4	0	49.7	60.2	7.92	4.27	7.24
	Slow	21.2	10	277	1.9	0	58.3	59.0	7.75	1.14	7.20
3	Initial unfrozen	–	176	73	12.2	10.0	30.4	58.3	6.80	5.75	7.40
	Rapid	0.23	174	128	11.6	5.2	34.2	57.1	6.67	5.37	6.90
	Intermediate	4.06	69	181	6.5	4.7	37.0	56.1	6.60	4.75	6.88
	Slow	19.3	14	254	0	0	49.6	54.7	6.28	1.29	6.75
4	Initial unfrozen	–	575	95	6.0	6.7	36.7	51.9	7.11	5.35	–
	Rapid	0.22	508	147	4.5	3.1	41.3	56.6	6.95	5.45	–
	Intermediate	6.15	388	227	5.0	0.8	40.2	54.5	6.74	4.64	–
	Slow	21.2	131	474	1.8	0	45.7	52.8	6.36	1.93	–
5	Initial unfrozen	–	147	93	10.7	3.9	35.5	53.3	7.63	5.69	7.30
	Rapid	0.23	96	115	10.8	2.7	36.8	50.5	7.21	5.57	7.20
	Intermediate	5.71	52	135	9.0	1.0	40.7	51.1	6.41	3.57	7.20
	Slow	21.5	7	252	2.8	0	47.6	47.5	6.57	0.96	7.10
6	Initial unfrozen	–	297	108	6.6	3.8	39.7	58.5	7.40	5.06	7.15
	Rapid	0.36	–	–	–	–	–	–	–	–	7.0
	Intermediate	5.97	118	273	5.3	0	43.3	54.0	6.60	3.65	7.0
	Slow	19.4	16	424	0	0	54.2	54.4	7.05	1.21	6.8
7	Initial unfrozen	–	396	105	8.3	5.0	34.4	56.8	6.66	5.31	–
	Rapid	0.25	323	156	7.0	2.9	37.1	56.8	6.50	5.10	–
	Intermediate	4.20	278	205	5.6	1.1	39.2	56.9	6.32	3.94	–
	Slow	14.5	123	379	1.5	0	44.6	56.0	6.29	1.77	–

[a] Twenty transverse slices of muscle (approx 7.8 × 1.5 × 1 cm) were cut from one fish immediately post mortem and grouped into four lots of five slices each. One lot of slices (unfrozen) was analyzed within 40 min post mortem to give initial values, whereas the other three lots were frozen sandwich style at rapid, intermediate, and slow rates as shown in Fig. 2. After freezing, the samples were either analyzed immediately or held at –26 C for not longer than 48 hr prior to analysis; experiments showed that no changes in metabolite levels occurred during this holding period. Each sample for investigation was composed of four slices and the fifth slice was used for *p*H determination. This experiment was repeated seven times on a total of seven fish. Fish 1 to 3 were immobilized in the aquarium by anaesthetic (Fraser et al., 1966) to eliminate struggling prior to death. These individuals had higher initial CP values than the unanaesthetized fish.

[b] The following abbreviations are used: TAT, thermal arrest time; CP, creatine phosphate; IP, inorganic phosphorus; TP, total phosphorus; TN, total adenosine and inosine compounds; and AN, adenosine compounds.

[c] Δ7P less CP represents acid-labile P chiefly from ATP and it is expressed as μg-at P/g of muscle. CP values for fish 1 and 4 were obtained by the procedure of Ennor and Rosenburg (1952) whereas in the other fish CP was determined as the increase in IP after hydrolysis in 0.1 N HCl at 100 C for 1 min.

TABLE 2. Amount of metabolites remaining in cod muscle after freezing at different rates, mean of the seven fish reported in Table 1.

Freezing rate	TAT[a] (*hr ± SE*)	% of initial value ± SE			
		Glycogen	CP	Δ7P less CP	AN
Rapid	0.22±0.03	84±4.6	61±5.1	89±3.7	96±2.3
Intermediate	5.40±0.34	50±3.4	15±6.7	71±4.9	73±3.5
Slow	19.9±0.99	13±3.9	0	13±4.9	26±3.0

[a] Abbreviations as in Table 1.

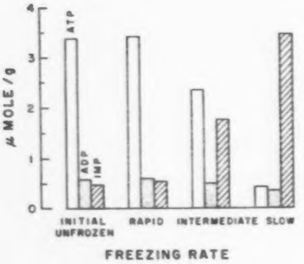
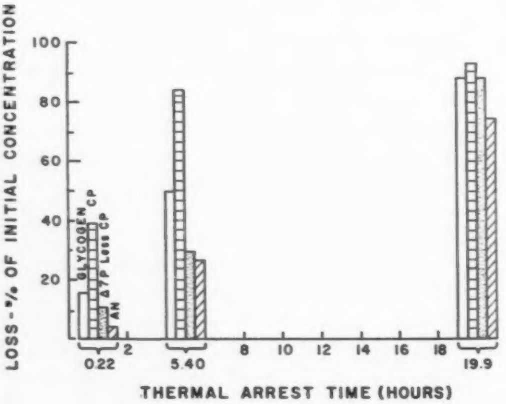


FIG. 3. Nucleotides in prerigor cod muscle before and after freezing at different rates. Results were obtained from TLC analysis of a neutralized perchloric acid extract of fish 7 in Table 1. TATs were 0.25, 4.2, and 14.5 hr for rapid, intermediate, and slow freezing rates, respectively.

FIG. 4. Relation between TAT and net loss of glycogen, CP, and nucleotides during freezing at three different rates. These data were obtained by subtracting the values in Table 2 from 100%; they represent an average of results from seven fish with initial individual glycogen values ranging from 150 to 600 mg/100 g.



Our results agree with the observation of Fraser et al. (1965) that losses of 20–70% of the initial glycogen and 25–60% of the Δ7P occurred in rested cod muscle during slow freezing in an ice–brine mixture for 4–5 hr. Partmann (1961) found that half of the initial ATP was destroyed in carp and rainbow trout muscle frozen under commercial conditions, and also that after blast freezing only 12% of rosefish and cod fillets contained 50% or more of the initial ATP. On the other hand, Tomlinson et al. (1963) detected no change in Δ7P in lingcod during freezing at −10 C, but this was probably because he used very small samples, which would have a relatively short TAT (although the actual rate of freezing was not measured).

To determine what proportion of the total biochemical activity during freezing occurs in the critical temperature zone, muscle samples were analyzed at various time intervals during freezing at intermediate and at slow rates. Losses of glycogen and Δ7P occurred mainly between 0 and −5 C during freezing at an intermediate rate (Fig. 5). Little further degradation occurred as the temperature continued to drop to the freezer temperature, −17 C, and metabolite levels were maintained during subsequent storage for 12 hr at −17 C. Likewise, in slowly frozen muscle the biochemical changes occurred mostly within the critical zone, and because of a longer TAT (15 hr for slowly frozen vs. 4 hr for muscle frozen at an intermediate rate) all changes had gone to completion by the time the sample had cooled to −5 C (Fig. 6). Some glycogenolysis occurred prior to freezing and may have been caused by physical stimulation

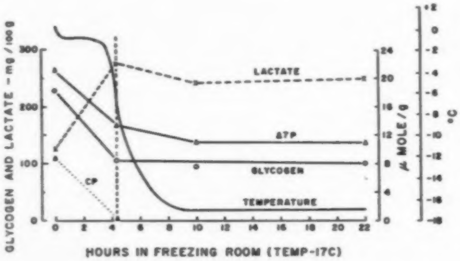


FIG. 5. Concentration of glycolytic and high-energy phosphorus metabolites in cod muscle during freezing at an intermediate rate (TAT, 4 hr). Muscle slices 1 cm thick were frozen in a sandwich pack (Fig. 1); each point represents an analysis of four slices of muscle taken together to form a composite sample. The zero time sample consists of unfrozen muscle analyzed at the time the other samples were placed in the freezer.

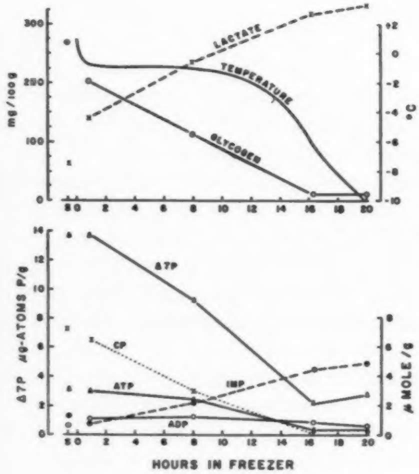


FIG. 6. Glycogenolysis and nucleotide catabolism in prerigor cod muscle during slow freezing at −10 C. *S*, an unfrozen sample for initial analysis was taken 30 min post mortem, and other samples from the same fish were placed in the freezer 55 min post mortem. Muscle slices were frozen in sandwich packs (Fig. 1) and each sample for analysis consisted of four slices.

as the muscle slices were cut and handled. Mechanical handling has been found to accelerate the breakdown of energy-rich nucleotides (Partmann, 1963).

The total amount of metabolic change is roughly proportional to time as the temperature falls from −0.8 to −5 C (approx 1–16 hr) (Fig. 6). Similarly, the extent of degradation increased with thermal arrest time when different freezing rates were studied (Fig. 4). The nearly linear curves (Fig. 6) suggest that the degradation rates are constant in the critical temperature zone, but confirmation of this observation by more frequent sampling is obviously desirable.

The results of such an experiment (Fig. 7) show that glycogen, ATP, and other metabolites have essentially a linear relationship with time, and that there is little apparent change in the reaction rates from the onset of freezing down to −2 C. However, most reactions were practically complete at −2 C so the data give no information on activity below this temperature.

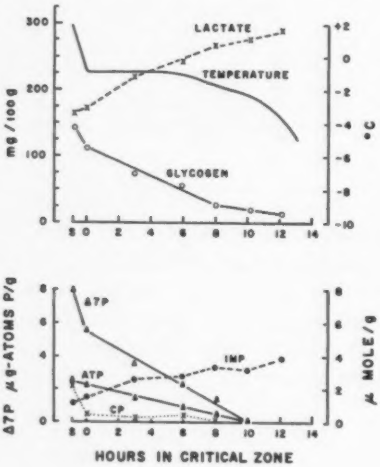


FIG. 7. Pattern of glycogenolysis and nucleotide catabolism in the critical freezing zone as the temperature drops from −0.8 to −4 C. *S*, an unfrozen sample was analyzed at the time that other samples from the same fish were placed in a freezing chamber at −10 C, 50 min prior to the first point in the critical zone. Samples were frozen sandwich style (Fig. 1) and two muscle slices were used for each determination.

Repetition of this experiment using two fish with higher initial glycogen concentrations revealed the same general pattern of degradation (Fig. 8) except that at temperatures below −1 C the rate of glycolysis appeared to increase and in one case (Fig. 8*b*) IMP formation was accelerated. No increase in glycolytic activity below −1 C was apparent in Fig. 7, probably because the glycogen concentration was almost depleted in the temperature plateau at −1 C.

Since the patterns of CP and Δ7P loss in Fig. 8*a* differed from those in Fig. 8*b* we were unable to determine if the rate of loss increased below −1 C. Part of the difficulty was caused by variations between duplicate samples, probably related to the condition of the original material. It is difficult to

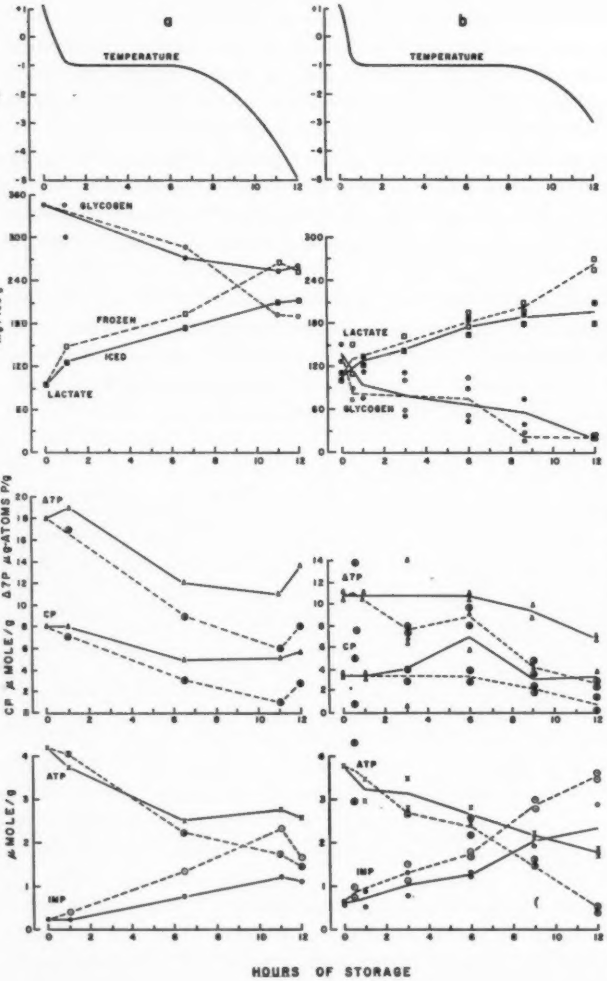


FIG. 8. Metabolic changes in prerigor cod muscle during slow freezing in the critical temperature zone compared with those in unfrozen muscle stored in ice. (*a*) Transverse slices of muscle 1 cm thick were cut from the midsection of fillets from a cod immediately post mortem and an initial analysis of the fresh muscle was made. Half of the slices were frozen sandwich style in a chamber at −10 C while the other half were placed in polyethylene bags and held in an ice bath at 0 C. Two slices were used for each determination. (*b*) Samples from another cod were analyzed more frequently; each point represents analysis of one muscle slice. Frozen muscle – – –; iced muscle ———.

obtain muscle samples (from one fish) containing the same amount of Δ7P because this metabolite is not distributed uniformly throughout the white muscle of unexercised cod and even adjacent samples can differ considerably (Fraser et al., 1966). Because of this variation and because the metabolites are nearly depleted by the time the temperature of the muscle falls below −1 C, further work on the temperature–rate dependence should be undertaken with the muscle brought quickly to the desired temperature below −1 C.

Degradation in Freezing Muscle at –1 C Compared with that in Unfrozen Muscle at 0 C

The rates of glycogen loss and lactate formation in partially frozen muscle at –1 C are comparable to those in unfrozen muscle from the same fish at 0 C (Fig. 8). However, as noted above, there appears to be an increase in rate at temperatures below –1 C, in the region of –1.2 to –4 C.

Loss of CP and Δ7P is more extensive during slow freezing (–1 C) than in iced muscle, a result in agreement with findings of Heen (1954). The patterns of CP and Δ7P are somewhat variable (Fig. 8), but the concentrations in frozen muscle are less than in the corresponding iced muscle at all sampling times.

Since Δ7P is derived mostly from ATP and CP, and changes in the Δ7P concentration give an indication of adenosine triphosphatase activity, our results show that this enzyme has greater activity in freezing muscle at –1 C than in unfrozen muscle at 0 C. Deamination of adenine nucleotides to IMP occurs more rapidly at –1 C (slow freezing) than at ice temperature (Fig. 8).

DISCUSSION

From the inception of freezing at –0.8 C, the temperature of prerigor cod muscle remains on a plateau at about –1 C for a considerable time. At very slow freezing rates glycogen and high energy phosphorus compounds are almost completely metabolized in this interval at a nearly constant rate; at the same time approximately 10% of the muscle water gradually crystallizes into ice (Dyer, 1968). At –1 C the apparent rate of breakdown of high energy phosphorus compounds is greater than in unfrozen muscle at 0 C. When the temperature drops below –1 C an acceleration in glycolytic activity occurs as the amount of water frozen out increases rapidly to 80% at –5 C.

As water is removed from muscle as pure ice crystals, the concentration of salts and metabolites increases, the *p*H changes as buffering salts crystallize out, viscosity increases, and proteins are denatured (see Zaitsev, 1965). Such changes can affect enzyme activity and probably are responsible for the accelerated reaction rates during freezing at –1 C and on down to –5 C.

The activity of glycolytic enzymes in both mammalian and fish muscle is increased by local environmental changes attendant upon ice formation. Cori (1965) reported that freezing stimulated conversion of phosphorylase *b* to the active *a* form in rabbit muscle, and Scopes and Newbold (1968) observed increased phosphofructokinase activity in frozen ox sternomandibularis; both of these enzymes are of prime importance in the glycolytic scheme. Our results from cod muscle indicate that the rate of glycogenolysis is accelerated at temperatures just below –1 C; it seems that all of the enzymes in the glycolytic scheme are stimulated during freezing since the amount of glycogen lost is comparable to the increase in lactic acid. In haddock an increase in glycolytic activity occurs around –2 C with maximum lactate formation at –3.5 C (Sharp, 1935). In cod muscle further work is needed to confirm whether a similar peak exists.

The rate of ATP breakdown is accelerated as ice develops in the tissue. In cod, ATP is dephosphorylated and deaminated to IMP more rapidly at -1 C (slow freezing), at which point about 10% of the water is frozen, than in unfrozen muscle at 0 C (Fig. 8), a result in agreement with findings of Saito and Arai (1957) for carp muscle. Similarly, Partmann (1963) found that the rate of ATP breakdown increased at -1 C as freezing commenced in carp and rainbow trout. The stimulatory effect of freezing on adenosine triphosphatase activity was illustrated by Bito and Amano (1960) who held frozen and unfrozen carp muscle at -2 C and found that ice formation enhanced the destruction of ATP whereas the unfrozen samples resisted degradation. Thus it is shown that increased enzyme activity is not caused by subzero temperature per se but rather by the freezing out of water and resulting changes in the tissue environment. It is possible that accelerated ATP breakdown during freezing is due to activation of adenosine triphosphatase by an increase in the Ca ion concentration as water is frozen out (Bito and Amano, 1960).

The extent of glycolytic and nucleotide changes in prerigor cod muscle during freezing depends largely on the time required for the sample to cool through the critical temperature zone from -0.8 to -5 C where most of the water is frozen out. Studies on board a freezer-trawler have shown that blocks of muscle 6 cm thick require 22 min to freeze from 0 to -5 C in a plate freezer (Meyer et al., 1967); when the TAT is this short, biochemical changes during freezing are trivial so that the frozen prerigor muscle may retain sufficient ATP and glycogen to support thaw rigor (if the muscle used had high initial metabolite levels). When larger and thicker samples are frozen under commercial conditions, the TAT is prolonged to several hours (MacCallum et al., 1965); biochemical changes will then be extensive and the muscle mass may no longer be in prerigor condition by the time it is frozen.

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Muscle Protein Polymorphism of Sablefish from the Eastern Pacific Ocean

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Muscle proteins of 897 specimens of sablefish, collected during 1963–67 in the northeastern Pacific Ocean, ranging from Unalaska Island, Alaska, to Cape Blanco, Oregon, were analyzed by starch gel zone electrophoresis. Without exception, all specimens fitted into one of three phenotypic protein patterns resulting from two polymorphic proteins appearing singly or in pairs. Hereditary control by two codominant alleles, Mu^A and Mu^B, is postulated to explain the heterogeneity of the muscle protein patterns. Phenotypic distribution was shown to be independent of age and sex. The collection was arbitrarily divided into 10 areas, and the gene frequencies used as population parameters suggested homogeneity but did not provide positive evidence of heterogeneity in the sablefish inhabiting the waters of the eastern Pacific Ocean. The implications of these results are discussed in the light of previously reported racial and tagging studies.

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INTRODUCTION

Available information on the movements of sablefish (*Anoplopoma fimbria*) in the Pacific northwest is not extensive, despite the commercial value of the species. In the early 1950's, tagging experiments were carried out by certain research agencies in California, Oregon, and Washington at the instigation of the Pacific Marine Fisheries Commission for the ultimate purpose of determining whether this species represents a single, large, freely migrating stock, or a series of localized stocks with limited intermingling. The results of that study (Holmberg and Jones, 1954), based on an inexplicably low overall tag return of 2.55%, indicated that most of the recoveries were within 30 miles of the point of tagging, although one fish was recovered 450 miles north and another 315 miles south of the tagging locations. Isolated examples of extensive southward migration from Cape Cleare off Alaska to Cape Flattery off Washington, a distance of 1230 miles, were reported by Edson (1954), and a reciprocal northward migration of 1100 miles from Holmes Harbor in Puget Sound to Middleton Island in the Gulf of Alaska was reported by Pruter (1959). Three fish resulting from the tagging program at Holmes Harbor were recovered approximately 2700 miles north in the Bering Sea, 6–7 years later, by the Japanese fishery (Pasquale, 1964). It appears, therefore, that although many of the tags were recovered within short distances of the point of tagging, sufficient recoveries were made at great distances to suggest intermingling of stocks in the range studied.

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The only existing racial study, based on meristic analyses and carried out jointly by fisheries agencies of Alaska, Canada, Washington, Oregon, and California on specimens from Cape Spencer in Alaska to Newport Beach in California, suggests the existence of four regional stocks (Phillips et al., 1954). These are (*a*) southern Alaska coast from Cape Spencer to Petersburg, (*b*) southern British Columbia and the northern Washington coasts, (*c*) central Washington coast to the northern California coast, and (*d*) the central and southern California coasts.

Electrophoretically demonstrable, genetically linked, polymorphic blood proteins are being used increasingly as biochemical characters in the stock analyses of fishes. In this connection, the Atlantic cod, *Gadus morhua*, has been studied with respect to both its serum transferrins (Møller, 1966, 1968) and its blood hemoglobins (Sick, 1965; Frydenberg et al., 1965). The serum transferrins, however, have been the protein group of choice and have been investigated in the gadoids (Møller and Naevdal, 1966), the scombroids (Barrett and Tsuyuki, 1967; Fujino and Kang, 1967), and in several other species. The protein group of choice is largely governed by the feasibility of sampling, the clarity of electrophoretic separations, and other factors.

The present investigation employs muscle proteins, first observed in clearly defined polymorphic forms during a routine screening of electrophoretic characters of marine fishes (Tsuyuki et al., 1965) in the population analysis of sablefish. Although the blood hemoglobins of this species also displayed polymorphisms of interest, the inconvenience of immediate electrophoretic analyses largely influenced the decision against its use at this time.

MATERIALS AND METHODS

SOURCE OF MATERIAL

Approximately 900 specimens of sablefish ranging from Unalaska Island, Alaska, to Cape Blanco, Oregon (Fig. 1, Table 1), were collected over 1963–67 aboard the *G. B. Reed* during groundfish cruises 63-7, 64-8, 65-3, 66-2 (Westrheim, MS, 1967), and 67-1 (Harling et al., MS, 1967), and the Investigator No. 1 groundfish cruise 64-12. All specimens were trawl caught. The collections over the 5-year period were divided arbitrarily into 10 areas, A to J, from the Aleutian Islands eastward to the Gulf of Alaska and southward to the Oregon coast (Table 1).

In about half the specimens fork length, sex, and age were determined. Sex was determined by macroscopic gonadal examination and age by annuli examination of scales removed from an area between the lateral line and the origin of the first dorsal fin (Kennedy and Pletcher, 1968).

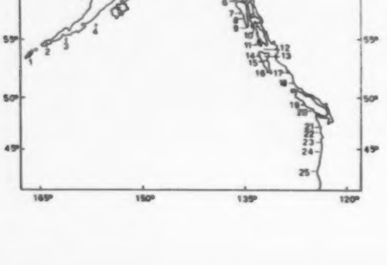

FIG. 1. Reproduction of the northeastern Pacific Ocean, from which sablefish were collected during 1963–67. Numbers 1–25 represent the collection areas in the order listed in Table 1.

TABLE 1. Sampling data, area designation, and distribution of phenotypes in sablefish collected from the eastern Pacific Ocean during 1963–67.

Area designation	Location	No. observations	Muscle protein phenotypes		
			AA	BB	AB
A	Unalaska Island	10	1	3	6
	Unimak Island	6	–	3	3
	Unga Island	6	–	3	3
	Semidi Island	6	1	4	1
	Kodiak Island	9	3	1	5
Subtotal		37	5	14	18
B	Cape Cross	16	2	4	10
	Kruzof Island	38	5	17	16
	Sitka Sound	36	6	11	19
Subtotal		90	13	32	45
C	Cape Ommaney	9	–	3	6
	Iphigenia Bay	23	2	14	7
	Forrester Island	38	7	13	18
	Dixon Entrance	10	1	3	6
Subtotal		80	10	33	37
D	W. Coast Graham Island	92	13	40	39
	Port Chanal	20	3	7	10
	Skidegate Channel	20	5	11	4
	Gowgaia Bay	40	6	17	17
Subtotal		172	27	75	70
E	Goose Island Grounds	156	30	63	63
F	Smith Sound	39	4	17	18
G	Estevan Point	60	6	24	30
H	La Perouse Bank	141	23	54	64
I	Grays Harbor	24	3	8	13
	Willapa Bay	9	3	3	3
	Tillamook Bay	29	3	9	17
Subtotal		62	9	20	33
J	Heceta Bank	36	4	16	16
	Cape Blanco	24	4	6	14
Subtotal		60	8	22	30
Grand total		897	135	354	408

Tissues were sampled from the lateral muscle from the midsection of the fish shortly after its capture and kept under frozen storage at –20 C until electrophoretic analysis could be carried out at a shore-based laboratory.

ELECTROPHORESIS

The red muscle tissue was cut away and only the white muscle was used for preparing extracts by a method described previously (Tsuyuki et al., 1965). The proteins were separated by starch gel electrophoresis in 0.023 M sodium borate buffer, *p*H 8.5, with 0.3 M sodium borate of the same *p*H in the bridge buffer solution, in an apparatus described previously (Tsuyuki et al., 1966).

RESULTS

Electrophoretically, all specimens analyzed showed muscle protein patterns that fitted into one of three types (Fig. 2). Considering only the region of the electropherogram displaying polymorphic proteins, all three types of patterns could be obtained by a variation of two protein zones, A and B, singly or as a pair, giving three phenotypes, AA, BB, and AB.

The distribution of the phenotypes in collections from the 10 areas are shown in Table 2. Chi-square tests for the individual areas — their sums, as well as the pooled collections — indicate no significant deviation between the observed and expected values, calculated from the Hardy-Weinberg proportions, in any of the 10 different areas, although the samples from the Goose Island grounds approach the 5% level of significance. Hereditary control by two codominant alleles, Mu^A and Mu^B, is thereby indicated. The samples from the 10 different areas do not show differential selection by phenotypes.

The distribution of phenotypes by length is shown in Table 3. Samples from the entire range of investigation were pooled and selected according to the fork lengths indicated, as only approximately half the specimens were measured, and as the number of samples was insufficient from most of the collection areas to analyze all six size ranges independently. Chi-square tests indicated that in all six size ranges the observed values for phenotypic distribution are in good agreement with those calculated according to the Hardy-Weinberg law. Likewise, no trend in phenotypic excess or deficiency was evident from the observed /expected ratios of phenotypes with size in contrast to that observed in the serum transferrins of skipjack tuna (Fujino and Kang, 1967). Chi-square tests for independence of phenotypes and size (Table 4) indicate that the incidence of observed phenotypes AA, BB, and AB in each of the six size ranges are in good agreement with the proportions calculated from the total sample. Both the individual chi-squares and their sums fall well above the 5% level of significance.

The influence of sex on the distribution of phenotypes in the whole range of collection was also determined (Table 5). The number of samples sexed as well as the totals sampled was insufficient in most of the 10 different collections for an independent analysis by area. The chi-square tests indicate that in either sex, the observed phenotypes are distributed in Hardy-Weinberg proportions. In tests for independence of phenotypes and sex (Table 6), both the individual chi-squares as well as their totals fall well above the 5% level of significance, indicating good agreement between the observed incidence of phenotypes in each sex and the expected values calculated from the proportions observed in the combined samples.

The frequency of the dominant gene, Mu^B, ranged from a low of 0.589 in area I to a high of 0.667 in area F (Table 7) and no particular east to west or north to south trend was indicated. The confidence limits of all collections were found to overlap one another.

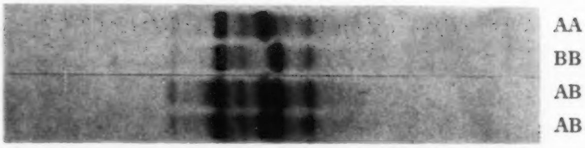

FIG. 2. Starch gel electropherograms of the muscle proteins of sablefish showing the three phenotypes observed. The anode is to the right and the cathode to the left.

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TABLE 2. The distribution of phenotypes by area.

Collection area	No. samples	Phenotypes						X^2	Probability of worse fit (1 df)
		AA		BB		AB			
		Obs.[a]	Exp.	Obs.	Exp.	Obs.	Exp.		
A	37	5	5.295	14	14.297	18	17.405	.043	.900–.750
B	90	13	14.004	32	32.994	45	42.984	.196	.750–.500
C	80	10	10.152	33	33.144	37	36.688	.005	.950–.900
D	172	27	22.343	75	70.348	70	79.292	2.367	.250–.100
E	156	30	24.242	63	57.236	63	74.506	3.724	.100–.050
F	39	4	4.333	17	17.332	18	17.332	.058	.900–.750
G	60	6	7.350	24	25.350	30	27.300	.587	.500–.250
H	141	23	21.446	54	52.452	64	67.088	.300	.750–.500
I	62	9	10.484	20	21.489	33	30.020	.609	.500–.250
J	60	8	8.814	22	22.812	30	28.356	.199	.750–.500
								$\Sigma 8.089$ for 9 df =	.500–.250
Pooled	897	135	128.092	354	347.049	408	421.769	.961 for 1 df =	.500–.250
								$X^2_{\text{hetero (9 df)}} = \Sigma X^2 - X^2_{\text{total}} = 8.089 - .961 = 7.128$	

[a] Obs. = Observed; Exp. = expected.

TABLE 3. The distribution and observed/expected ratios of phenotypes by size.

Fork length (*cm*)	Total obs.[a]	Phenotypes						Frequency		X^2	Probability of worse fit (1 df)	O/E ratios of phenotypes		
		AA		BB		AB								
		Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	q^A	q^B			AA	BB	AB
<30	41	6	5.486	17	16.486	18	19.024	.366	.634	.119	.750–.500	1.094	1.031	.946
31–40	62	9	7.111	29	27.106	24	27.764	.339	.661	1.144	.500–.250	1.266	1.070	.864
41–50	74	9	10.974	26	27.972	39	35.046	.385	.615	.940	.500–.250	.820	.930	1.113
51–60	49	6	5.557	22	21.550	21	21.883	.337	.663	.080	.900–.750	1.080	1.021	.960
61–70	144	23	23.357	51	51.350	70	69.264	.403	.597	.016	.900–.750	.985	.993	1.011
>71	57	6	6.002	26	26.003	25	24.989	.325	.675	.000		1.000	1.000	1.004
Overall	427	59	58.072	171	170.074	197	198.726	.369	.631	.035	.900–.750	1.016	1.005	.991

[a] Obs. = observed; Exp. = expected; O/E = observed/expected.

TABLE 4. Chi-squares for independence of phenotypes and size.

Phenotype		Fork length (*cm*)						Total	X²	Probability (5 df)
		<30	31–40	41–50	51–60	61–70	>71			
AA	Obs.[a]	6	9	9	6	23	6	59	1.203	.950–.900
	Exp.	5.665	8.566	10.224	6.770	19.896	7.875			
BB	Obs.	17	29	26	22	51	26	171	2.664	.900–.750
	Exp.	16.419	24.829	29.634	19.622	57.667	22.826			
AB	Obs.	18	24	39	21	70	25	197	1.844	.900–.750
	Exp.	18.915	28.604	34.140	22.606	66.435	26.297			
Total		41	62	74	49	144	57	427	5.711[b]	.990–.975

[a] Obs. = observed; Exp. = expected.
[b] 15 df.

TABLE 5. Phenotypic distribution by sex.

		Phenotype									
		AA		BB		AB					
Sex	Total	Obs.[a]	Exp.	Obs.	Exp.	Obs.	Exp.	q^A	q^B	X²	Probability of worse fit (1 df)
Male	190	27	25.403	78	76.418	85	88.122	.366	.634	.244	.750–.500
Female	268	44	45.131	92	93.130	132	129.658	.410	.590	.084	.900–.750

[a] Obs. = observed; Exp. = expected.

TABLE 6. Chi-square for independence of phenotypes and sex.

Phenotypes		Sex		Total	X^2	Probability (1 df)
		Male	Female			
AA	Obs.[a]	27	44	71	.349	.750–.500
	Exp.	29.454	41.545			
BB	Obs.	78	92	170	1.353	.250–.100
	Exp.	70.524	99.475			
AB	Obs.	85	132	217	.478	.500–.250
	Exp.	90.021	126.978			
Total		190	268	458	2.180[b]	.990–.975

[a] Obs. = observed; Exp. = expected.
[b] 3 df.

TABLE 7. The Mu^B gene frequency in the 10 samples.

Collection area	No. genes in sample	Observed frequency Mu^B allele	95% confidence limit of Mu^B frequency
A	74	.622	.511–.732
B	180	.606	.534–.677
C	160	.644	.570–.718
D	344	.640	.573–.696
E	312	.606	.552–.660
F	78	.667	.562–.771
G	120	.650	.565–.735
H	282	.610	.553–.667
I	124	.589	.502–.675
J	120	.617	.530–.704

DISCUSSION

It has been established that the polymorphic muscle proteins of sablefish in this investigation are under genetic control and that neither size nor sex of the fish introduces selective differences in the distribution of muscle protein phenotypes. The gene frequencies observed and used as a population parameter did not provide positive evidence for the existence of separate stocks suggested by the earlier study (Phillips et al., 1954), which recognized, on the basis of meristic analyses, three regional stocks in specimens collected over a geographic range overlapping with the present investigation in the area from Cape Spencer to the Oregon coast. The fourth regional stock off the coast of California, suggested by that study, was not included in the present investigation. The extensive migrations observed in tagging studies indicate, however, that considerable gene flow may occur in sablefish inhabiting the waters of the northeastern Pacific Ocean. It is also possible that geographic selection of this species, if any, may not revolve around the genetic system involved in these particular muscle proteins. It is important, therefore, that several other genetic systems, such as those involved in the synthesis of blood hemoglobins and serum transferrins, be investigated for a more extensive evaluation of the population structure of sablefish in these areas.

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Discussion of Calculation of Power Function with Special Reference to Respiratory Metabolism in Fish

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GLASS, NORMAN R. 1969. Discussion of calculation of power function with special reference to respiratory metabolism in fish. J. Fish. Res. Bd. Canada 26: 2643–2650.

The rationale for employing a nonlinear iterative least-squares technique for fitting the well-known power function to oxygen consumption–body weight data is set forth. Twenty-six sets of routine or standard metabolism data from six authors were used to demonstrate the relative merits of two methods of calculating parameter values for the power function. The conclusion was reached that if accuracy in predicting oxygen consumption over a wide range of values of body weight is desired, an iterative curve fitting method may be superior to the much used technique of performing a linear regression on logarithmically transformed data.

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INTRODUCTION

FOR MANY YEARS, physiologists — including fisheries biologists interested in respiratory metabolism in fish — have been collecting data on oxygen consumption for different weights of fish at different temperatures. The most common way of expressing the functional relationship between oxygen consumption and body weight has been to employ the power function, or allometric equation as a descriptor. To be sure, the simple equation

$$Y_i = a\ X_i^b \tag{1}$$

where Y_i = oxygen consumption, X_i = body weight, and a and b are fitted parameters, has a reasonably well-documented theoretical basis and also appears to fit oxygen uptake vs. body weight data consistently. The equation itself is fairly easy to understand, and it may readily be transformed by simply taking logarithms of both sides:

$$\ln Y_i = \ln a + b \ln X_i. \tag{2}$$

However, it is in this transformation and the standard linear regression that invariably follows that a statistical error of varying proportion may be committed. That error of this type might occur has apparently been noted by only one biologist (see Zar, 1968). Zar points out that a log–log transformation of equation 1 and the subsequent least-squares regression to determine parameter values may only provide crude estimates of the true or best parameter values, and frequently the approximation is poor. Although equations 1 and 2 are

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equal mathematically, statistical equivalence for least-squares solution may not be true. In the case of the power function or any other functional relationship considered, an understanding of the way in which random error (ϵ_i) enters the equation is of critical importance. If, as is the case in some biological relationships, the error term is multiplicative e.g.,

$$Y_i = [f(x,\beta)]\epsilon_i \tag{3}$$

then a logarithmic transformation is not only possible, but is quite correct for a least-squares determination of parameter values since

$$\ln Y_i = \ln [f(x,\beta)] + \ln \epsilon_i. \tag{4}$$

On the other hand, if ϵ_i enters the equation additively — a situation that also arises in biological data — then the following relationship is pertinent

$$Y_i = f(x,\beta) + \epsilon_i. \tag{5}$$

It is in this circumstance that a logarithmic transform is invalid and an alternative procedure for estimation of parameters is in order. The question that must be answered, therefore, before any parameter estimation is attempted revolves about the nature of the variance observed in Y_i. The assumption that underlies all of the following discussion is that equation 5 is true. The only measure of the validity of this assumption lies in the *raw* data of each investigator; the *mean* values obtained from the literature from which Table 1 was derived do not provide the information necessary to confirm either equation 3 or equation 5. However, since situation 5 represents for biologists a very probable and somewhat more difficult case, a detailed exposition would appear to be valuable.

In fitting a line to a set of data by least squares, the object is to find parameter values that minimize the residual sum of squares $\Sigma (Y_i - \hat{Y}_i)^2$. Ordinarily this is accomplished by solving two or more linear equations simultaneously as follows. For the two-parameter linear equation $Y_i = a + b\,X_i$ (which resembles equation 2 in general form)

$$\Sigma R_i^2 = \Sigma(Y_i - \hat{Y}_i)^2 = \Sigma(Y_i - a - b\,X_i)^2. \tag{6}$$

Then, taking partial derivatives of ΣR_i^2 with respect to a and b and setting these equal to zero,

$$\frac{\partial \Sigma R_i^2}{\partial a} = -2\Sigma(Y_i - a - b\,X_i) = 0 \tag{7}$$

$$\frac{\partial \Sigma R_i^2}{\partial b} = -2\Sigma X_i(Y_i - a - b\,X_i) = 0 \tag{8}$$

and rearranging and summing as indicated, the well-known normal equations are obtained

$$\begin{aligned} na + b\,\Sigma X_i &= \Sigma Y_i \\ a\,\Sigma X_i + b\,\Sigma X_i^2 &= \Sigma X_i Y_i. \end{aligned} \tag{9}$$

From equation 9, *b* and, subsequently, *a* are easily determined. Notice, however, that if equation 2 is used as the linear model, the quantity that is minimized is not $\sum_{i=1}^{n} (Y_i - \hat{Y}_i)^2$, but rather $\sum_{i=1}^{n} (\ln Y_i - \widehat{\ln Y_i})^2$. It is here that the statistical inequality of equations 1 and 2 is most in evidence. Minimizing $\Sigma(\ln Y_i - \widehat{\ln Y_i})^2$, in effect, gives differential weight to those points nearest the origin, i.e., higher weight to those points that represent small body weight–oxygen consumption data. In short, when a log–log transformation is made on respiratory metabolism data, the measurements on smaller fish are given greater importance than measurements on large fish in the determination of both *a* and *b*. This means that calculations of the exponent in equation 1 by linear regression with 2 are suspect, and are actually dependent upon the range of body weight over which data were collected, and upon the scatter in the raw data. If the scatter in the data is small and the weight range over which measurements were taken is also small, then estimates of *a* and *b* by linear regression of $\ln Y_i$ on $\ln X_i$ will be reasonably close to true values of *a* and *b*. If the weight range over which data are collected is large, and the error follows condition 5, then *a* and *b* determined by linear regression of $\ln Y_i$ on $\ln X_i$ would be expected to differ more widely from the best values of *a* and *b*.

Clearly, if the accuracy of estimating *a* and *b* for equation 1 is important, then the data must be used in the untransformed state, and the parameter values in equation 1 determined by some method other than the log–log transformation. So, if the quantity analogous to equation 6,

$$\Sigma R_i^2 = \Sigma(Y_i - \hat{Y}_i)^2 = \Sigma(Y_i - a X_i^b)^2, \tag{10}$$

is to be minimized for a least-squares solution, the following partial derivatives of ΣR_i^2 with respect to *a* and *b* should be taken and set equal to zero

$$\frac{\partial \Sigma R_i^2}{\partial a} = 2\Sigma(Y_i - a X_i^b)(X_i^b) = 0 \tag{11}$$

$$\frac{\partial \Sigma R_i^2}{\partial b} = -2\Sigma(Y_i - a X_i^b)(a X_i^b \ln X_i) = 0. \tag{12}$$

Notice that two normal equations in two unknowns cannot be derived from equations 11 and 12. Fortunately, it is possible to determine true values of *a* and *b* by other means.

There are three primary techniques in current use for fitting data to equations with parameters that enter nonlinearly (e.g., *b* enters equation 1 nonlinearly). The three techniques are: the Taylor series expansion method where successive corrections to parameter estimates are found until convergence to true, or best, parameter values is achieved (see Glass, 1967); Gradient Methods (see Spang, 1962); and a method developed by Marquardt (1963), which combines the best features of both of the above. These three techniques have been illustrated, and their relative merits discussed elsewhere (Conway et al., MS, 1969), so no detail about iterative least-squares regression will be given.

RESULTS AND DISCUSSION

If a log–log transformation gives estimates of *a* and *b* that are inaccurate compared to *a* and *b* determined by an iterative least-squares method, how great is the disparity? By what criterion is the latter method judged superior to the former? Table 1 provides one possible answer to both questions. Data from several authors for various fish species were obtained from the literature. Equation 2 was fitted to all 26 data sets by taking logs of oxygen consumption (mg O_2/hr) and body weight (g). All data sets that were obtained and tested appear in Table 1 — no data were discarded. A standard linear regression was performed on the transformed data, and best parameter estimates for this method were computed. Since a linear regression of $\ln Y_i$ on $\ln X_i$ yields the slope (*b*) directly, this value appears in column 6. This regression, however, gives $\ln a$ as the intercept, so $e^{\ln a} = a$ is shown in column 5. Equation 1 was also fitted to all 26 sets of data by Marquardt's algorithm as set forth by Conway et al. (MS, 1969). Both *a* and *b* are computed directly (columns 9 and 10). Thus, parameter estimates determined by the log–log transformation method (columns 5 and 6) may be compared directly to parameter estimates found by the iterative least-squares technique (columns 9 and 10). A comparison of columns 6 and 10 reveals that a discrepancy exists between the parameter values as they are determined by the two methods and that the difference is frequently sizeable (i.e., 10% or more). Furthermore, it may be noticed, not surprisingly, that if the value for *b* determined by, say, the log transformation method is larger than *b* determined by the iterative least-squares method, then *a* estimated by the log transformation technique will be smaller than the iterative least-squares estimate of *a*. Also, a row-by-row comparison of the residual sum of squares that result from the two fitting methods (columns 7 and 11) points out their relative capabilities for producing parameter values that yield the best fit of equation 1 to the data. In every case, the iterative least-squares method gives parameter estimates that produce a smaller residual sum of squares than the log transformation technique. Occasionally, under rather specialized circumstances, it might be desirable to judge the goodness of fit of an equation to a set of data by a different criterion than achieving a minimum sum of squares of deviations between observed and calculated values of the dependent variable. However, in most instances the residual sum of squares of deviations provides a rational basis for evaluating goodness of fit. Therefore, if goodness of fit is judged on the basis of obtaining a minimum residual sum of squares, then Table 1 provides convincing arguments for using iterative nonlinear least-squares fitting techniques for determining best parameter values for equation 1.

To reinforce this point, the first set of data in Table 1 (that of Wells, 1935) was plotted on an arithmetic scale (see Fig. 1). The parameter values both the usual log–log transformation and a nonlinear fitting routine. Columns 8 and 12 show the values calculated for Student's t-test and at the bottom of columns 6 and 10 the weighted mean values for each b column appear.

Reference	Species	Temp (C)	n[a]	Log transformation fit a	b	$\Sigma (Y_i - \hat{Y}_i)^2$	t	Iterative least-squares fit a	b	$\Sigma (Y_i - \hat{Y}_i)^2$	t
Column: 1	2	3	4	5	6	7	8	9	10	11	12
Wells (1935)[b]	Killifish	16	21	0.2583	0.5931	0.033	0.435	0.2427	0.6442	0.021	0.445
Beamish and Mookherjii (1963)[b]	Goldfish	10	13	0.0165	0.9659	5.758	0.003	0.0077	1.1179	4.852	0.006
"	"	20	13	0.0724	0.7919	29.606	0.005	0.0317	0.9796	2.651	0.002
"	"	30	13	0.2708	0.6938	23.980	0.008	0.1699	0.7929	2.030	0.006
"	"	35	14	0.2530	0.8133	279.250	0.002	0.0242	1.2605	105.442	0.011
Brett (1965)	Salmon	15	6	0.2218	0.7884	177.066	<0.001	0.7609	0.6170	117.380	0.001
Beamish (1964)[b]	Brown bullhead	10	14	0.0049	1.2822	3.171	0.025	0.0055	1.2664	3.052	0.020
"	" "	20	14	0.1028	0.9007	28.524	0.001	0.1859	0.7879	25.203	0.007
"	" "	30	16	0.1933	0.8625	77.667	0.001	0.2238	0.8381	75.942	0.006
"	Carp	10	16	0.0214	0.9724	28.162	0.003	0.0627	0.7837	25.449	0.004
"	"	20	16	0.0837	0.8945	55.181	<0.001	0.0492	0.9968	45.814	0.002
"	"	30	13	0.2338	0.8644	87.544	0.001	0.2796	0.8349	84.700	0.003
"	Common white sucker	10	14	0.0498	0.9212	15.491	0.003	0.0675	0.8635	15.293	0.005
"	" " "	15	16	0.1631	0.8340	87.200	0.002	0.1334	0.8838	81.850	0.003
"	" " "	20	12	0.4173	0.7048	28.784	0.008	0.3727	0.7307	27.866	0.009
"	Brown trout	10	11	0.1202	0.9227	348.018	0.001	0.0829	0.9954	340.361	0.002
"	" "	10	14	0.0392	1.1529	61.510	0.013	0.0207	1.2812	54.113	0.017
"	" "	15	13	0.0881	1.0634	562.658	0.007	0.0251	1.3146	469.103	0.015
"	" "	20	15	0.1170	1.0555	46.143	0.011	0.0793	1.1434	21.562	0.013
Glass (1969) table 1	Largemouth bass	12	5	0.3964	0.6618	17.525	0.006	0.1286	0.9034	8.027	0.001
"	" "	20	5	0.5167	0.6294	0.530	0.033	0.4207	0.6954	0.413	0.032
"	" "	25	6	0.4019	0.7785	0.228	0.012	0.4916	0.7155	0.115	0.025
Job (MS, 1954)[c] App. table V	Brook trout	5	6	0.0363	0.8338	1.711	<.001	0.0150	0.9724	0.7385	<0.001
"	" "	10	6	0.0715	0.8447	0.107	<.001	0.0629	0.8650	0.097	0.001
"	" "	15	6	0.1068	0.8372	0.180	<.001	0.1134	0.8269	0.018	0.001
"	" "	20	6	0.1499	0.8038	0.022	0.001	0.1471	0.8067	0.007	0.001
					$\bar{b} = 0.8803$				$\bar{b} = 0.9382$		

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[a] n = number of data points used. In most cases, the number of individuals or observations pooled was greater than n.

[b] Data estimated from graphs.

[c] Data from Job (MS, 1954) were converted from cc/hr to mg/hr oxygen consumption.

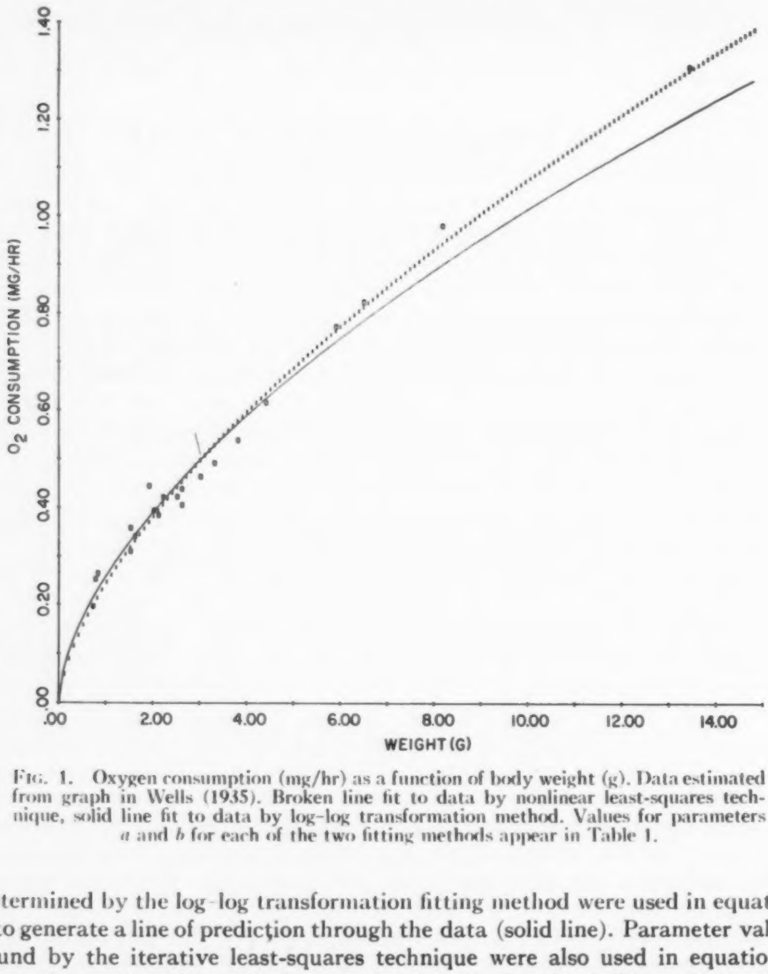

FIG. 1. Oxygen consumption (mg/hr) as a function of body weight (g). Data estimated from graph in Wells (1935). Broken line fit to data by nonlinear least-squares technique, solid line fit to data by log-log transformation method. Values for parameters *a* and *b* for each of the two fitting methods appear in Table 1.

determined by the log-log transformation fitting method were used in equation 1 to generate a line of prediction through the data (solid line). Parameter values found by the iterative least-squares technique were also used in equation 1 to produce a line of best fit through the data (broken line). Figure 1 therefore provides a visual aid for evaluating the relative merits of the two methods of fitting data and may serve to persuade the skeptical reader to investigate nonlinear curve fitting techniques. The same function was used to compute the residual sum of squares — only the parameter values differed. It should also be clear that the log-log transformation will produce parameter values that fit the data closest to the origin best, and those points representing larger body weights will fall relatively far from the calculated line of best fit as can readily be seen in Fig. 1. If the range of body weight to which equation 1 is fitted is made smaller, the same observation will hold. Ultimately, of course, the range of body weight to which equation 1 is fitted may become so small that only two data points are included. In that case, both fitting techniques will give the same parameter estimates and the same residual sum of squares (the predicted line will of course be a straight line between the two points). In all except this limiting case, the iterative least-squares fit to the data will yield a better line of prediction, assuming that the residual sum of squares is the criterion used for goodness of fit.

A weighted mean value of b ($\bar{b}$) for all data sets and each fitting method is given at the bottom of columns 6 and 10 of Table 1. These weighted mean values were used in a computation of Student's t-test to test the hypothesis that none of the individual values for b, determined by each fitting method for each of the 26 data sets, was significantly different from the weighted mean value for b. It is obvious that the values for t, which appear in columns 8 and 12, are very small and, statistically, the estimated values for b determined by both fitting methods did not differ from the appropriate $\bar{b}$ at the .05 level of significance. This would suggest that any grouping of values for b in equation 1 by species of fish or by acclimation temperature (cf. Fry, 1957) may well be the result of abberations in the data. This does not mean that no physiological differences between species or acclimation temperatures exist, merely that if they do, it has not been adequately demonstrated. In all likelihood such variations in oxygen uptake may reasonably be expected, but variability in the data is so great that no more than the suggestion that the exponent, b, in equation 1 varies in a real way is warranted.

Therefore, it would seem reasonable to submit that in fitting the power function to data collected on oxygen consumption, nonlinear iterative methods should be generally preferred. This is particularly true if the numerical or relative value of the exponent is of critical importance, or if statistical arguments are used to demonstrate a conclusion that is based upon the parameter values for the power function. FORTRAN IV listings of either the Taylor series or the Marquardt method are available from the author. The Marquardt method is also available as an IBM SHARE program.

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Effect of EDTA Treatment on Spoilage Characteristics of Petrale Sole and Ocean Perch Fillets

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PELROY, GRETCHEN A., AND JOHN P. SEMAN, JR. 1969. Effect of EDTA treatment on spoilage characteristics of petrale sole and ocean perch fillets. J. Fish. Res. Bd. Canada 26: 2651–2657.

Ethylenediaminetetraacetic acid (EDTA) used in 1% solution as a dip extended the 0.5-C shelf life of petrale sole and ocean perch fillets by repressing growth of *Pseudomonas* spoilage organisms and formation of trimethylamine. Of the three salts compared, $\mathrm{Na_2EDTA}$ and $\mathrm{Na_4EDTA}$ were the most effective, extending shelf life of ocean perch fillets by 7–10 days, whereas $\mathrm{Na_2CaEDTA}$ gave a 4-day extension over the control fillets. Vacuum packaging enhanced the effectiveness of EDTA as a preservative.

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INTRODUCTION

VARIOUS SODIUM SALTS of ethylenediaminetetraacetic acid (EDTA) have been shown to be effective as dipping solutions for extending the storage life of raw fish (Levin, 1967; Boyd and Southcott, 1968; Power et al., 1968). The mechanism for the preservative effect of EDTA was not established in these studies. All of the investigators found that EDTA treatment did not affect significantly the rate of increase of the total bacterial population on the fish throughout storage, although trimethylamine (TMA) formation was repressed. Levin (1967) demonstrated that $\mathrm{Na_4EDTA}$ in nutrient broth inhibits the growth of certain *Pseudomonas* species associated with fish spoilage, but in work with fish he found no obvious shift in predominating spoilage species.

This paper presents observations on the storage-life characteristics of fish fillets treated with $\mathrm{Na_2EDTA}$, $\mathrm{Na_2CaEDTA}$, and $\mathrm{Na_4EDTA}$ stored under both air and vacuum, and evidence that the change in the spoilage pattern of the treated fish is related to a shift in the predominating spoilage microflora.

METHODS

Fillets of Pacific ocean perch (*Sebastodes alutus*) were dipped in $\mathrm{Na_2EDTA}$, $\mathrm{Na_4EDTA}$, and $\mathrm{Na_2CaEDTA}$ and sealed (with and without vacuum) in laminated mylar-polyethylene pouches. Organoleptic evaluations were made on both the raw and cooked fish. The samples were scored for organoleptic quality by a panel of experienced judges, using a 10-point rating scale with a mean overall score of 5 indicating spoilage (Miyauchi, 1964). Fillets of petrale sole (*Eopsetta jordani*) were dipped only in $\mathrm{Na_4EDTA}$. They were then packed in both oxygen-permeable polyethylene bags and oxygen-impermeable, laminated mylar-polyethylene bags. The fillets in the oxygen-impermeable bags were vacuum sealed. Organoleptic evaluations of the petrale sole fillets were scored from the raw state on the basis of odor.

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The TMA nitrogen content of the fillets was measured using the method of Dyer (1945). EDTA determinations were done using the method of Groninger and Brandt (1969). The methods used for the total bacterial plate count and identification of the microflora have been described (Pelroy and Eklund, 1966).

RESULTS

SHELF LIFE

Vacuum-packaged petrale sole fillets treated with Na_4EDTA were of borderline acceptability after 9 days of storage and were characterized by stale and acetic odors. The control samples had reached advanced spoilage with putrid and ammoniacal odors after 7 days. The air-packaged petrale sole that had been treated with Na_4EDTA was at the borderline of acceptability after 6 days. The controls were definitely spoiled, as characterized by putrid and ammoniacal odors.

In the experiments with ocean perch, vacuum packaging resulted in an extension of shelf life over the nonvacuum-packaged fish in both the treated and untreated samples. This extension ranged from 1 day for the controls and the $Na_2CaEDTA$-treated samples to 4 days for the Na_4EDTA-treated samples (Table 1).

Of the three EDTA salts tested, $Na_2CaEDTA$ gave the least extension of shelf life: 4 days vs. 7–10 days for the Na_2EDTA and Na_4EDTA. Treatments with Na_2EDTA and Na_4EDTA were equivalent in storage-life extension when the samples were vacuum stored, but Na_2EDTA gave slightly better shelf life than Na_4EDTA for the nonvacuum-packaged samples.

BACTERIAL CHANGES

Quantitative — Vacuum-packaged petrale sole that was Na_4EDTA-treated had slightly lower bacterial counts throughout storage than the untreated samples (Fig. 1). In the air-packaged petrale sole, total bacterial counts were similar in the treated and untreated samples. At the time of spoilage of the untreated fish (6 days), the counts were 9.7×10^6 and 2.0×10^6 bacteria per gram for the untreated and treated fish, respectively.

The total bacterial population on the untreated ocean perch was the same as on the treated fish initially and grew at the same rate throughout the storage period. The untreated ocean perch spoiled when the bacterial count was in the range of 5.8×10^5 to 2.5×10^6 bacteria per gram, but the treated fish did not become spoiled until the count had reached 3.3×10^6 to 7.5×10^7 bacteria per gram (Table 1).

Qualitative — The initial bacterial flora (Table 2) of the untreated petrale sole was composed of predominantly *Achromobacter* (36–65%). The numbers of *Pseudomonas* (4–6%) and lactic acid bacteria (0–3%) isolated were small. The composition of the flora of the fillets was relatively unchanged immediately after treatment with Na_4EDTA.

During storage at 0.5 C, the microflora of the untreated, vacuum-packaged petrale sole became predominantly *Pseudomonas* and lactic acid bacteria. The

TABLE 1. Spoilage characteristics of vacuum- and nonvacuum-packaged ocean perch treated with various salts of EDTA[a] and stored at 0.5 C.

	Vacuum-packaged				Nonvacuum-packaged			
			Bacterial population at spoilage				Bacterial population at spoilage	
Treatment	Days of storage until spoilage	Mg/100 g TMA nitrogen at spoilage	*Pseudomonas* (%)	No. bacteria/g	Days of storage until spoilage	Mg/100 g TMA nitrogen at spoilage	*Pseudomonas* (%)	No. bacteria/g
Control	6	22.24	99	5.8×10^5	5	16.96	90	2.5×10^6
$Na_2CaEDTA$	10	6.00	34	8.7×10^6	9	2.61	10	3.3×10^6
Na_2EDTA	16	1.68	29	3.7×10^6	14	2.44	2	2.1×10^7
Na_4EDTA	16	2.20	14	3.1×10^7	12	1.51	4	7.5×10^7

[a] EDTA, ethylenediaminetetraacetic acid; TMA, trimethylamine.

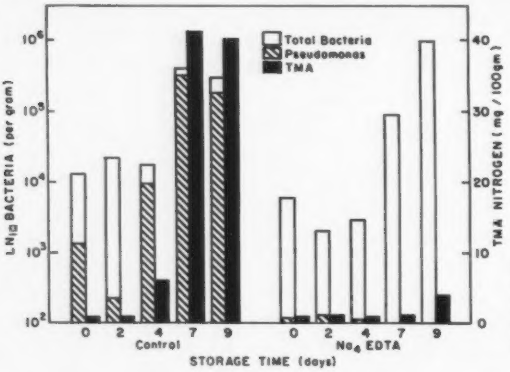

FIG. 1. Total bacterial counts, total *Pseudomonas* counts, and TMA levels in EDTA-treated and untreated vacuum-packaged petrale sole fillets throughout storage at 0.5 C.

microflora of the air-packaged fish became predominantly *Pseudomonas* and *Achromobacter*. Further characterization of the *Pseudomonas* organisms showed that they were all *P. putrifaciens*. The proportion of the other organisms that had comprised the initial flora showed no increase.

Throughout storage, the microflora of the treated petrale sole differed from that of the controls in both the air- and vacuum-packaged samples. In the treated fish the percentage of *Pseudomonas* did not increase. In the air pack, the predominant flora at the time of spoilage was *Achromobacter*. In the vacuum pack, the spoilage flora consisted of coliforms (40%), lactic acid bacteria (37%), and *Achromobacter* (19%). None of the coliforms were of fecal origin.

In the experiments with ocean perch only the *Pseudomonas* portion of the bacterial flora was identified (Table 1). In the treated fish, *Pseudomonas* comprised 4–16% of the population initially, and this proportion did not change appreciably until the last days of storage. The proportion of *Pseudomonas* on the control fillets increased rapidly from 5 to 26% initially until, at spoilage, they comprised 90–99% of the total population

The total numbers of *Pseudomonas* at the time of spoilage were, in general, similar for the treated and untreated fish. The main difference was in the length of time required to reach that level.

TRIMETHYLAMINE NITROGEN

TMA nitrogen content was consistently lower in EDTA-treated fish than in untreated fish stored under the same conditions. In vacuum-packaged, Na_4EDTA-treated petrale sole the TMA value was 0.65 mg/100 g initially and did not increase until the 9th day of storage, when 3.5 mg/100 g were found (Fig. 1). In contrast, the value for the control fillets was 42.0 mg/100 g by the 7th day.

In the nonvacuum-packaged petrale sole, the TMA levels in the treated samples went from 3.5 mg/100 g initially to 15.3 mg/100 g after 6 days of storage.

TABLE 2. Generic distributions of the microflora from vacuum-packaged and air-packaged Na_4EDTA-treated and untreated petrale sole fillets throughout storage at 0.5 C.

			Isolates (%)							
Treatment	Days of storage	No. isolates	*Pseudo-monas*	*Achromo-bacter*	Lactic acid bacteria	Coliforms	Coryne-forms	*Micro-coccus*	Other	Not identified
					Vacuum-packaged					
Control	0	69	6	36	3	13	26	11	3	2
	2	39	2	43	3	0	36	8	0	8
	4	66	42	30	6	3	14	2	0	3
	7	91	93	1	3	0	1	1	1	0
	9	104	70	1	16	8	1	1	2	1
Na_4EDTA	0	117	2	26	6	2	36	4	2	22
	2	41	7	25	5	7	37	2	5	12
	4	74	5	26	3	18	32	4	7	5
	7	90	1	23	32	28	0	11	2	3
	9	161	0	19	37	40	0	0	2	2
					Air-packaged					
Control	0	72	4	65	0	0	15	4	5	7
	3	55	58	20	11	0	2	0	2	7
	6	48	61	27	4	0	4	0	0	4
	8	58	64	21	2	0	1	2	5	5
Na_4EDTA	3	98	7	56	6	0	6	2	1	22
	6	106	15	72	3	0	0	0	0	10
	8	95	4	83	1	0	0	0	0	12

TMA values at the time of spoilage of the ocean perch also showed a suppression of TMA formation by EDTA (Table 1).

EDTA Content of Fillets

The mean EDTA content of the nonvacuum-packaged ocean perch fillets at the time of sampling was 305 ppm for the Na_4EDTA-treated fish, 396 ppm for the Na_2EDTA-treated fish, and 411 ppm for the $Na_2CaEDTA$-treated fish. At present, the U.S. Food and Drug Administration allows only the sodium calcium salt of EDTA to be added to seafoods: cooked canned clams, 340 ppm; cooked canned crab meat, 275 ppm; and cooked canned shrimp, 250 ppm.

DISCUSSION

Our conclusion is that the increased shelf life in the EDTA-treated fillets is due to the inhibition of growth and concomitant metabolic activity of *Pseudomonas*. In the control fillets the rise in *Pseudomonas* population was associated with a rise in TMA content whereas in the treated fillets the *Pseudomonas* population was depressed and so was the formation of TMA even though the rate of increase of the total population was not affected.

Evidence that *Pseudomonas* is the source of TMA production in spoiling fish has been found previously in studies with radiation-pasteurized petrale sole fillets (Miyauchi et al., 1966). In vacuum-packaged, nonirradiated fish, the predominant spoilage organism was *Pseudomonas*, and TMA content was high — in the range of 30–40 mg/100 g. In fillets irradiated at 0.2 megarad and above, however, *Pseudomonas* was virtually eliminated (Pelroy and Eklund, 1966; Pelroy et al., 1967), and TMA levels were never found to go above 7.0 mg/100 g even after as long as 6 weeks of storage at 0.5 C. If after irradiation the fillets were inoculated with *Pseudomonas*, these organisms increased in numbers, and putrid and ammoniacal spoilage odors and high levels of TMA nitrogen were produced.

The results of this study agreed generally with those of previous workers in showing that the $Na_2CaEDTA$ salt was the least effective in increasing the shelf life of the fillets. Na_4EDTA and the Na_2EDTA were almost equal in effect. The difference in effectiveness of the three salts is probably due to the fact that Na_4EDTA and Na_2EDTA can sequester calcium as well as cations with higher stability constants than calcium, whereas $Na_2CaEDTA$ does not remove additional calcium from the system. Calcium and magnesium ions are necessary for the maintenance of structural integrity of many gram-negative bacteria, and their removal from the cell membrane can cause lysis of the cell (Brown and Richards, 1965). The sensitivity to EDTA of *Pseudomonas* in general (Wilkinson, 1967), and species associated with fish spoilage in particular (Levin, 1967), has been documented.

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Preliminary Investigation of the Exploitation of Some Potential Nutritional Resources by Three Sympatric Tubificid Oligochaetes[1]

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Eight heterotrophic aerobic bacteria were identified in samples from Toronto Harbour. The three tubificid species present seem to ingest most if not all of these, but different species survive passage through the gut. One of the three worm species absorbs amino acid from solution in the absence of bacteria in the gut. It is suggested that the results indicate differences in the utilization of the potential nutritional resources by the worms, which may be reflected in the ability of three unspecialized sediment feeders to coexist in the same microhabitat.

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Huit bactéries hétérotrophes ont été recueillies dans le port de Toronto. Les trois espèces de Tubicifides présentes, semblent ingérer la plupart de ces bactéries, sinon toutes. Cependant, différentes espèces survivent après leur passage à travers les intestins. Une de ces trois espèces de vers absorbe un acide aminé en solution, même en l'absence de bactéries dans l'intestin. Il semble que les résultats indiquent des différences dans l'utilisation des resources potentielles de nutrition par les vers. Ceci peut être démontré par la capacité de ces trois mangeurs de sédiment nonspécialisés, de coéxister dans le même microhabitat.

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INTRODUCTION

IN DISCUSSING the results of a detailed study of the distribution of tubificids in Saginaw Bay, Lake Huron, it was concluded that "with... few exceptions, the distribution of tubificid species cannot be discussed in terms of the ecological factors commonly measured. Some more critical evaluation of the type of food material available in the sediments may be required to establish habitat preferences for these worms" (Brinkhurst, 1967). Much the same opinion had already been expressed by Brinkhurst and Kennedy (1965) as a result of a field and laboratory study of factors determining the coexistence of three

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[1] Based on a thesis submitted by K.E.C. in partial fulfillment of the requirements for the degree of Doctor of Philosophy from the University of Toronto, Toronto, Ont.

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species in a small stream in England. In the same study and many other investigations into the distribution of tubificids in a single microhabitat, several species were found to be intimately associated spatially over long periods of time. The mechanism by which several unspecialized detritus feeders are able to coexist also seems likely to involve the food resources utilized by each species from the common pool.

A series of studies of the distribution of tubificids in the St. Lawrence Great Lakes have been made in recent years, and these have been summarized by Brinkhurst et al. (1968). These studies suggest that a series of associations of species can be recognized, each of which occupies regions of the lakes at a similar stage of eutrophication or pollution (using the former to mean changes in the whole ecosystem generated by "the process of enrichment with nutrients" — Stewart and Rohlich, 1967 — and the latter to mean the reduction in diversity in the biotic community brought about by addition or accumulation of material at a rate inimical to the existence of at least part of the biota). One such species association of tubificids is that composed of *Tubifex tubifex* Müller, *Limnodrilus hoffmeisteri* Claparede, and *Peloscolex multisetosus* (Smith). In the St. Lawrence Great Lakes this association is found in areas polluted by excessive amounts of organic matter, of which Toronto Harbour is a good example. This association was chosen for detailed study for several reasons. The species may be separated readily using only a low-power stereo microscope, which makes it possible to work with live specimens in pure culture. Toronto Harbour is close to the laboratory in which the work was to be carried out, and offers an extensive area of sheltered water underlain by soft sediments containing large numbers of worms. Samples can be obtained on a year-round basis as part of the harbour is kept ice-free by aeration during the winter.

The study consisted of (1) identifying the bacteria that could be cultured aerobically on nutrient agar plates; (2) the utilization of these by the three tubificid species present; (3) determining the ability of worms to take up glycine (as shown by autoradiography) from solution under inhibition of the intestinal microflora.

METHODS

Samples of mud and worms were obtained using a newly developed corer (now obtainable from Wildlife Supply, Michigan, under the name KB corer — see Brinkhurst et al. (1969) for details of the instrument and its use). Samples of mud were placed in sterile containers and returned to the laboratory. A series of cultures were made with sterile worm saline (NaCl, 6 g; KCl, 0.12 g, $CaCl_2$, 0.2 g; $NaHCO_3$, 0.1 g; distilled H_2O, 1 liter) (Lockwood, 1963), a dilution of 1 part mud to 100 parts saline giving the most suitable number of colonies on the plates. Saline was used as the worms would not survive washing and immersion in distilled water for a week.[2] Also the dissections described below were carried out in saline. All of the bacteria obtained in samples from mud diluted with saline survived exposure to the saline for periods of a week and no growth inhibition was observed. After a variety of culture media and temperatures had been tested, most of the work was carried out using nutrient agar incubated at 25 C.

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[2] Lake water was not used because the worms inhabit the sediments rather than the water, and purifying the heavily polluted lake water would involve complex procedures. Laboratory conditions are very artificial in other respects anyway.

Some of the mud was screened in the field and five worms of each species were picked from each sample. These were killed in 5% NaCl solution. The dead worms were then transferred to containers previously filled with sterile saline. In the laboratory these worms were washed 10 times, with 5 ml sterile saline used for each wash. They were pumped in and out of each wash with a Pasteur pipette, carry-over being reduced by changing the pipette every few washes. The final wash water was plated to check on the efficacy of the washing procedure. The worms were then dissected with sterile needles in a petri dish. The contents of the foregut were mixed with 0.1 ml worm saline, and cultures of the mixture were incubated.

The residue of each sample containing live worms was also taken back to the laboratory. Five living specimens of each species were picked out and these were washed in the manner already described. They were then kept in capped test tubes with 5 ml sterile saline at 4 C. After 1 week the worms were removed. The faeces and saline were shaken to make a suspension, and cultures of this mixture were made. The worms themselves were washed again, and cultures of the gut contents were prepared as described above. Again the final wash water was plated.

Pure culture isolates of the bacteria obtained by these methods were obtained by repeated subculturing. Biochemical and morphological studies on the isolates were carried out according to the methods described by Bailey and Scott (1962), Breed et al. (1957), Cowan and Steel (1966), Gibbs and Skinner (1966), and Sneath (1960). The identifications were confirmed by an independent analysis carried out by the Bacteriology Branch of the Ontario Water Resources Commission.

The free amino acids in the sediment were extracted from separate samples brought to the laboratory in sterile containers. Wet mud (500 ml) was washed twice by vigorous shaking in a flask containing 200 ml of distilled H_2O. The mud was allowed to settle at 5 C after each wash. The supernatant was filtered through Whatman no. 1 paper and was centrifuged at 3020 × g (5000 rpm) for 15 min. The supernatant was sterilized by passing it through millipore filter (0.45 μ pore size). As a preservative, 1:10,000 merthiolate solution was added to the filtrate, which was then concentrated to about one four-hundredth of its original volume by flash evaporation at 35 C. A 10-ml concentrate of each sample was washed twice in cold chloroform to eliminate lipids. After shaking, the mixture was placed in a separatory funnel for 15 min, when the water soluble upper fraction was drawn into a 10-ml hypodermic syringe. A Swinny adaptor was then fitted to the syringe, supporting a metricel membrane (0.45 μ pore size) through which the solution was expressed into a sterile tube. The samples were held at 4 C until needed for qualitative analysis of the amino acids. This was carried out on one-dimensional descending paper chromatograms as described by Harrison et al. (1966).

The investigations of amino acid uptake were carried out using glycine-2-C^{14} (New England Nuclear — specific activity 2.89 mc/mmole). This is one of the more common amino acids isolated from the sediments and was readily available to us. Our primary objective in this initial study was to determine the possibility of direct uptake of amino acid from solution by any of the three species of worm studied in relation to differences in the nutritional physiology of the worms, which might help to elucidate the apparent failure of the competitive exclusion principle when applied to tubificid ecology. Live worms were sorted from field samples and were washed as described above. Half of them were used to investigate the uptake of amino acid in the presence of the normal gut microflora after the macroscopic gut contents had been allowed to evacuate normally for a week (a process that took longer for *P. multisetosus* and might not have been completed in specimens of that species — a point that may have some bearing on the results of the study). The rest of the living worms were placed individually in 5 ml of sterile saline containing 6300 units of polymixin. After 2 hr the worms were treated with a mixture of 25 μg neomycin and 7400 units of streptomycin in 5 ml saline.

Five hours later the worms were placed in 5 ml saline at 4 C. The next day, 0.1 ml of the suspension of the faeces in this saline was plated to check for the presence of bacteria. Half of the specimens of each species were killed and the gut contents plated as described above. In all instances the plates from faeces and gut contents of worms treated in this way failed to develop bacterial colonies, indicating that inhibition of the microflora of the gut had been achieved.

The exposure to labelled glycine was carried out by placing three worms of the same species in a tube containing 5 ml saline and 1.65 μc of glycine-2-C^{14} for 1 hr. The worms were fixed in 6% formalin overnight. Sections were cut at 4 μ and stained with eosin, and then autoradiographs were prepared by the technique described by Joftes (1963), with NTB_2 emulsion. The sections were exposed for 3 weeks in a dark dry-slide chamber at 5 C with drierite. They were developed in K-19 for 2 min and fixed.

RESULTS

Bacteria

Eight species of heterotrophic aerobic bacteria developed on nutrient agar plates, but only seven developed at any one time. There appears to be an alternation between *Chromobacterium* sp. and *Aeromonas* sp. (Table 1).

Table 1. Data showing the alternation between the appearance of *Chromobacterium* and *Aeromonas* in samples from the mud.

Sample data	*Chromobacterium*	*Aeromonas*
	1967	
Apr. 19	×	–
Apr. 28	–	–
May 10	–	–
May 31	–	×
June 5	–	×
June 13	–	×
June 20	–	×
Sept. 12	–	×
Sept. 26	×	–
Oct. 20	×	–
	1968	
Apr. 4	×	–
Apr. 16	×	–

When the gut contents of worms killed in the field were investigated, all seven species of bacteria were found in *P. multisetosus*. *Aeromonas* sp. was not found in either *T. tubifex* or *L. hoffmeisteri*, however, and *Pseudomonas fluorescens* was missing from *T. tubifex*. This result might suggest that, with the possible exception of *Aeromonas* sp., there was no sign of selective feeding, but a more detailed examination of the results on a quasi-quantitative basis further complicates the picture. The study was repeated on four occasions and each time five plates were prepared for each unit of the investigation (mud, worms killed in the field, worms after 1 week — three species of worms, i.e., seven units). As there were seven species of bacteria studied, the total number of positive identifications of bacteria for each unit of the investigation was 140 (four runs, five plates per run, seven species potentially present on each plate). Samples from the mud yielded the near-perfect total of 125 positive records, the scarcest bacterium being *P. fluorescens*, which was recorded on only 10 of the possible 20 plates. The total number of positive records of bacteria obtained from the guts of tubificids varied from 37 to 76 out of a possible 140, so that, despite the presence of between five and seven of the seven possible bacterial species, the microflora in the gut does not appear to be the same as that in the mud. The difference observed might be accounted for by selective feeding, especially in view of the records for *Aeromonas* sp. On the other hand, it is clear that these samples do not represent the microflora prior to digestion. When worms were sorted from mud in the field and returned to the laboratory alive, very few species of bacteria were found in the gut. Killing the worms in the field increased the spectrum of species found in the gut, presumably by preventing such digestion as occurred during transit to the laboratory. Careful investigation of ingestion in the living worm revealed intermittent periods of reverse peristalsis, which effectively mixed newly ingested food with partially digested material, so that a true reflection of ingestion is probably hard to obtain. Furthermore the technique of obtaining samples from the gut may lead to a greater dilution of the microflora than that employed in sampling mud.

Despite these short-comings, the difference in the microflora after 1 week was obvious, as was the consistent differences among the worm species. Only one bacterial species survived passage through the gut, a different species in each instance. *Aeromonas* sp., possibly ingested only by *P. multisetosus*, was the only bacterium to survive in that worm species. *Micrococcus* sp. and *Pseudomonas* sp. were well represented in the "ingestion" samples (32 out of 60, and 51 out of 60, respectively, and at about the same frequency for each tubificid species) but *Micrococcus* survived in *Limnodrilus* alone and *Pseudomonas* in *Tubifex*. Other bacteria, whether abundant or scarce in the "ingestion" pattern were completely absent after complete digestion.

A series of three investigations of the effect of passage of bacteria through *P. multisetosus* was made in April and May 1967, i.e., at a time when *Aeromonas* was absent. *Chromobacterium* was present only during the first of the three runs (April 19), and survived passage through the gut together with *P. fluorescens*. During the second and third runs all six species found in the mud were found in the guts of *Peloscolex*, but only *P. fluorescens* survived after 1 week. Again, a single species survived in the gut of the worm after a week (two species when *Chromobacterium* was present), but this time it is one of the species that disappear when *Aeromonas* is present.

AMINO ACIDS

Seven amino acids were detected in extracts from the sediments prepared as described above, of which glycine was apparently one of the most abundant (Fig. 1.). Although only insignificant uptake could be detected in *T. tubifex* and *L. hoffmeisteri*, a very heavy concentration of labelled glycine was demonstrable in *P. multisetosus* (Fig. 2). This labelled glycine was concentrated almost entirely in the chloragogen, a mass of cells that envelope the intestine of the worms, forming a sort of digestive gland in which protein metabolism is said to occur (Meglitsch, 1967). The glycine was taken up by specimens of *P. multisetosus* that had been exposed to antibiotics as well as by those that had not.

DISCUSSION

In this work the main emphasis has been on the qualitative analysis of the bacteria and amino acids that could be detected by standard microbiological procedures and the effect on these of passage through the gut of three species of tubificid oligochaetes. The results suggest that there are differences among the worms in regard to the uptake of one amino acid from solution, and the ability to digest at least some of the bacteria present.

No attempt has been made to measure the rate of utilization of any of these components of the sediment, or the utilization of other components such as carbohydrates, amino acids other than glycine, or anaerobic bacteria or the host of other possible food sources in the mud. Such studies will be pursued in future, of course, but the work completed to date constitutes the first demonstration of differences between tubificid species in regard to the way they exploit the nutritional resources of the environment. The success of these studies may be contrasted with the lack of success of earlier attempts to determine the mechanism by which anything from 2 or 3 to 14 tubificid species can be found in sediment from a single locality.

Most of the previous studies on nutrition of tubificids have involved measurements of the amount of faecal matter produced in relation to factors such as temperature, or changes in the total organic content of sediment after passage through the gut (Ravera, 1955; Poddubnaja, 1961; Wachs, 1967). Poddubnaja and Sorokin (1961) and Sorokin (1966) claimed to have demonstrated differences between *L. hoffmeisteri* adults and juveniles and *T. tubifex* in regard to the depth at which feeding is concentrated. Coler et al. (1967) showed that, under test conditions, worms react differently to various bacteria, which may indicate selective feeding, at least when the worms are presented with pure cultures of bacteria.

It is difficult to see how worms could select certain bacteria when faced with a mixture of species associated with sediment.

Sections of cores indicate that tubificids coexist vertically as well as horizontally in the mud (Brinkhurst and Kennedy, 1965; unpublished data), and they feed by ingesting the sediment as a whole. Most of the bacteria found in the mud can be found in the foregut of worms killed in the field (Table 2). If the worms are kept alive for a short time, one or two species disappear, and if they are kept for a week a single bacterium survives passage through the gut. The retention of *Micrococcus* by *L. hoffmeisteri* and of *Pseudomonas*

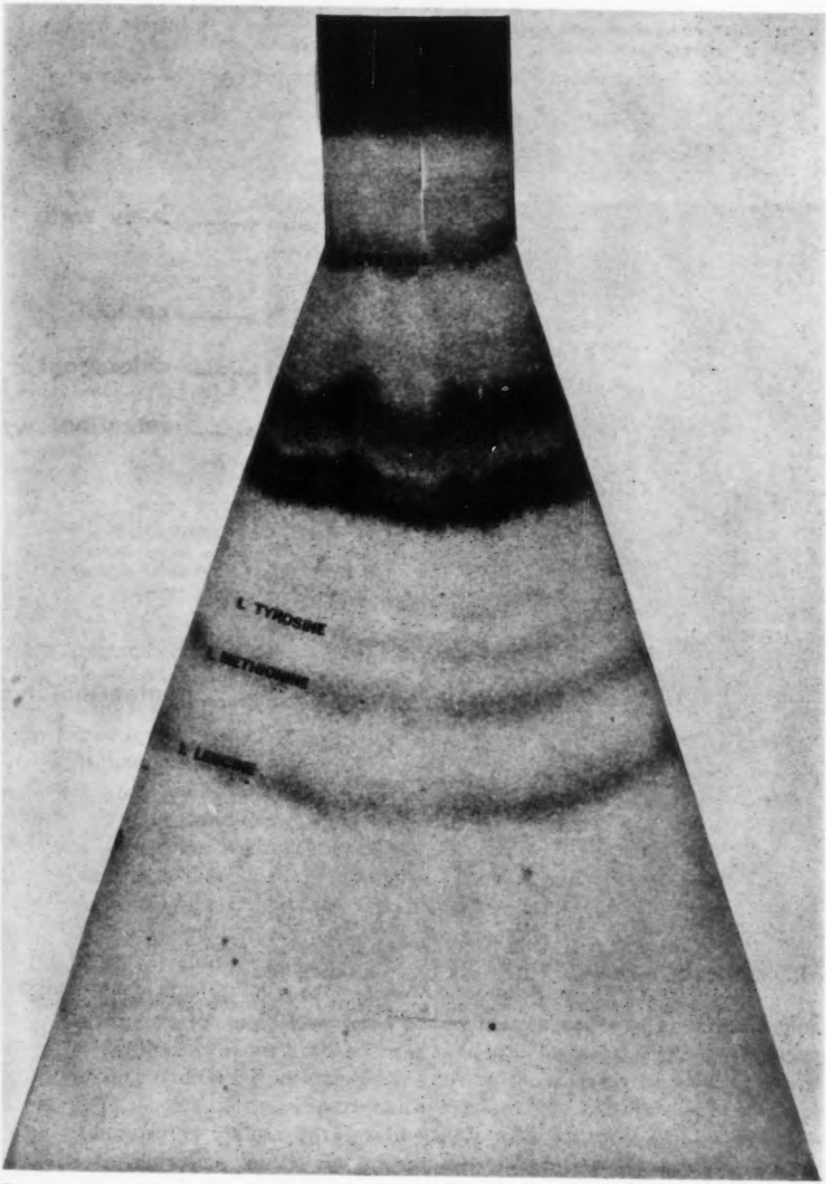
L TYROSINE
L METHIONINE
L LEUCINE

FIG. 1. Typical descending paper chromatogram showing amino acids separable from Toronto Harbour sediments.

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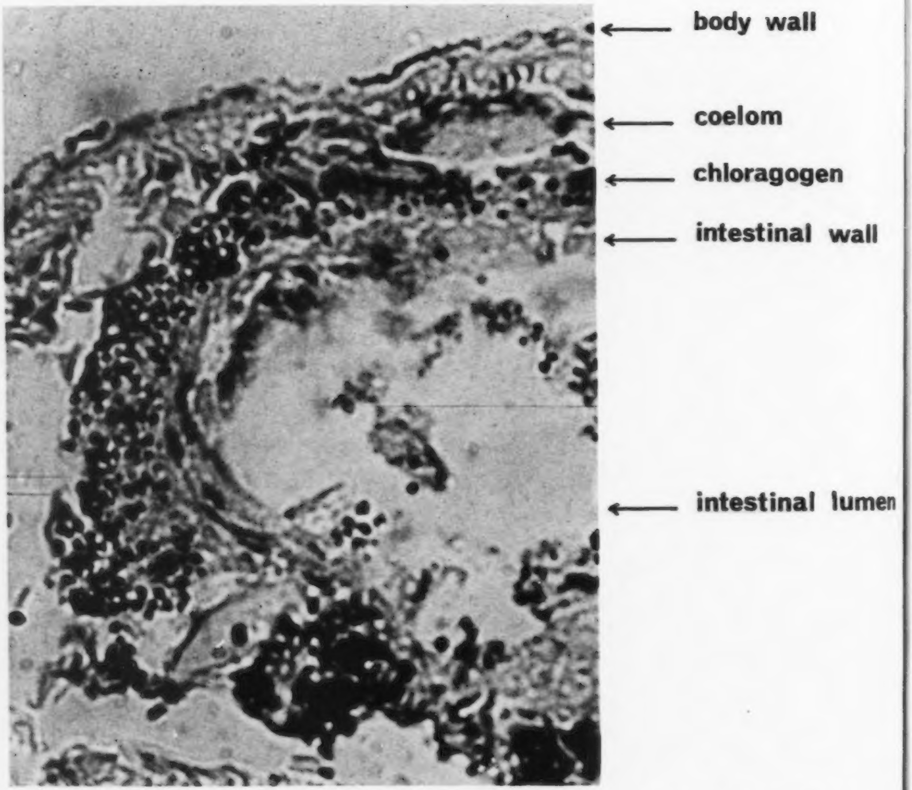
body wall
coelom
chloragogen
intestinal wall
intestinal lumen

FIG. 2. Autoradiograph showing accumulation of glycine-2-C^{14} in the chlorogogen tissue surrounding the gut of *Peloscolex multisetosus*.

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TABLE 2. Bacteria present in mud, in samples of worms killed in the field (after some digestion), and in worms starved for a week (complete digestion). Samples from Toronto Harbour on four dates in May–June 1967. Numbers represent number of plates showing positive growth.

Bacteria	No. mud samples	Samples from worms killed in the field			Samples from faeces and gut after 1 week		
		T.t[a]	*L.h*[a]	*P.m*[a]	*T.t*	*L.h*	*P.m*
Flavobacterium sp.	20	8	5	16	0	0	0
Pseudomonas fluorescens	10	0	2	5	0	0	0
Bacillus mycoides	19	2	1	1	0	0	0
Aeromonas sp.	20	0	0	14	0	0	20
Micrococcus sp.	16	10	12	10	0	15	0
Pseudomonas sp.	20	15	16	20	15	0	0
Bacillus cereus	20	2	4	10	0	0	0
No. of species present	7	5	6	7	1	1	1
Expected[b]	140	140	140	140	140	140	140
Observed[b]	125	37	40	76	15	15	20

[a] *T.t* = *Tubifex tubifex*, *L.h* = *Limnodrilus hoffmeisteri*, *P.m* = *Peloscolex multisetosus*.
[b] Expected based on 5 plates per run, 4 runs, 7 species recognized equals 140 possible positive records. Observed equals number of plates on which positive growth was observed.

by *T. tubifex* is clearly not due to selective ingestion. Failure to demonstrate the presence of *Aeromonas* in the gut of *Tubifex* or *Limnodrilus* does not constitute evidence of selective ingestion by *Peloscolex*, in which it is ingested and where it also survives after a week. The absence of *Aeromonas* in these two tubificids, and the scarcity or absence of bacteria such as *P. fluorescens*, *Bacillus mycoides*, and *Bacillus cereus* in the worms may be caused by failure to ingest them or by their rapid destruction in the gut of the worms. More sophisticated quantitative studies and tracer studies must be completed before this issue can be resolved. One final comment on this matter should be made. There seems to be no evidence from this study of the existence of a specific gut microflora, but if one wished to consider the bacteria that survive a 1-week exposure to the worm as constituting such a microflora, the ecological significance of the difference among the worm species still holds.

The observation that the number of species of bacteria present declined when worms were kept for a week in the saline might be attributed to progressive mortality caused by prolonged exposure to the salts in the solution. However, exposure of either each pure culture or a mixed culture of the bacterial species under investigation in the saline for a week failed to demonstrate any progressive mortality caused by the salt in the solution. The result of using three different worm species was that different species of bacteria survived in each instance, an observation difficult to tally with the concept of such progressive mortality.

TABLE 3. Bacteria present in mud, in guts of worms killed in the field, and surviving passage through the gut of *Peloscolex multisetosus*, April–May 1967. Numbers represent number of plates showing positive growth of each bacterium.

Bacteria	Mud samples	Samples from worms killed in the field	Samples from faeces and gut after 1 week
Flavobacterium sp.	15	15	–
Pseudomonas fluorescens	15	15	15
Chromobacterium sp.[a]	5	5	4
Bacillus mycoides	14	5	–
Micrococcus sp.	9	9	–
Pseudomonas sp.	15	15	–
Bacillus cereus	15	14	–
No. species present	7	7	2
Expected[b]	105	105	105
Observed	88	78	19

[a] All on first run only.

[b] Expected based on 5 plates per run, 3 runs, and 7 species recognized equals 105 possible positive records.

Recent studies on the uptake of amino acids by marine, brackish, and freshwater organisms by Stephens (1964) has reawakened interest in this subject, which was discussed earlier by Pütter (1909) and Krogh (1931). Although significant uptake of amino acids has been demonstrated for marine organisms, studies on freshwater organisms to date have suggested that they are unable to take up any significant amount. Part of the reason for this failure may be attributed to the selection of the 12 or so freshwater organisms used (which included *Lumbricus terrestris* but omitted most detritus-feeding macrobenthic forms such as tubificids). No explanation of the mechanisms by which *P. multisetosus* can take up such significant amounts of glycine from solution is available yet, but it should be noted that there was no noticeable bacterial activity in the gut of any of the worms exposed to the antibiotics, so that it would seem that the uptake observed was not mediated by the gut microflora.

Before making any attempt to interpret the results in terms of the natural situation, one or two points concerning the methodology should be emphasized. The use of a single culture medium (selected after comparative studies using a number of media), the use of saline (rather than the grossly polluted lake water), and the restriction of the study to heterotrophic aerobic bacteria (rather than extending the study to include anaerobes and other forms) certainly restricted the number of bacterial species studied. Similarly, the study of the uptake of amino acids was made using only glycine. This restriction does not affect the results obtained using this particular set of bacteria, amino acids, and worm species, but it does mean that it would be difficult to discuss the interpretation of the results in terms of their significance in the field. To identify factors responsible for the distribution or abundance, or both, of worms in the field, the study will have to be extended to include other possible nutritional resources in the mud, and the whole study will then have to be repeated at a number of locations inhabited by a variety of worm species. The gamut of experiments on the degree of dependence of each worm on certain components of the system will also have to be carried out. The present study is taken to indicate the value of pursuing this approach further, and is presented as a stimulus to further work on the true nature of animal to sediment relations. At present, such studies seem to rely heavily on the study of the inorganic constituents of sediment and the commonly measured limnological parameters.

The free organic matter and the microflora available as food for detritus feeders may prove to be more directly relevant to their ecology than the physical and inorganic chemical factors commonly investigated.

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Nutrition and Chemical Composition of the Rhabdocoel Turbellarian *Syndesmis franciscana*, with Notes on the Taxonomy of *S. antillarum*

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Mettrick, D. F., and J. B. Jennings. 1969. Nutrition and chemical composition of the rhabdocoel turbellarian *Syndesmis franciscana*, with notes on the taxonomy of *S. antillarum*. J. Fish. Res. Bd. Canada 26: 2669–2679.

Histological, histochemical, biochemical, and ultrastructural studies show that digestion in *Syndesmis franciscana* (Lehman, 1946), a rhabdocoel entocommensal in the coelom and intestine of the sea-urchin *Strongylocentrotus purpuratus* from California, is both extra- and intracellular, involving an endopeptidase, exopeptidases, phosphatases, and a lipase. The gastrodermal cells are loaded with lipid globules, so that this substance forms over 25% of dry weight. Glycogen accounts for a further 19% dry weight. Over 85% of the glycogen is in the soluble *lyo*-form, as in adult endoparasitic helminths. Both the form and total amount of glycogen present suggest that the pattern of carbohydrate metabolism is more akin to the parasitic than the free-living platyhelminths.

Syndesmis antillarum Stunkard and Corliss, 1951, a syndesmid from the Caribbean, is shown to differ both morphologically and physiologically from *S. franciscana*. The present synonymy of these two species is considered unjustified and incorrect.

Mettrick, D. F., and J. B. Jennings. 1969. Nutrition and chemical composition of the rhabdocoel turbellarian *Syndesmis franciscana*, with notes on the taxonomy of *S. antillarum*. J. Fish. Res. Bd. Canada 26: 2669–2679.

A l'aide des études histologiques, histochimiques, biochimiques et ultra-structurales, on a démontré que chez *Syndesmis franciscana* (Lehman, 1946), un rhabdocoele entocommensale dans le coelome et l'intestin de l'oursin de mer *Strongylocentrotus purpuratus* de Californie, la digestion est à la fois extra- et intracellulaire comprenant une endopeptidase, des exopeptidases, une lipase et des phosphates. Les cellules gastrodermiques sont chargées avec des globules lipidiques de façon à ce que cette substance forme plus de 25% du poids sec. Un supplément de 19% du poids sec est apporté par le glycogène. Plus de 85% de ce glycogène est sous une *lyo*-forme soluble comme chez les helminthes endoparasites adultes. Aussi bien la forme que la quantité totale du glycogène présent, laisse supposer que le modèle du métabolism des hydrates de carbones est plus proche des platelminthes parasites que des plateleminthes libres.

Entre *Syndesmis antillarum* Stunkard and Corliss, 1951, un syndesmide des Carraibes et *S. franciscana*, des différences aussi bien morphologiques que physiologiques ont été montrées. Le rapprochement actuel de ces deux espèces est considéré comme injustifié et incorrect.

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INTRODUCTION

LEHMAN (1946) described a parasitic turbellarian, *Syndisyrinx franciscanus*, from the intestine of the sea-urchin *Strongylocentrotus franciscanus* at Pacific Grove, California. Other rhabdocoels, stated by Lehman to be identical with *Syndisyrinx franciscanus*, but not described in detail, were obtained from the sea-urchin *Strongylocentrotus purpuratus*.

Marcus (1949) suppressed *Syndisyrinx* as a synonym of *Syndesmis*, a genus erected by Silliman (1881) for a rhabdocoel parasite from the sea-urchin *Echinus sphaera* found at Roscoff, France. This synonymy was accepted by Stunkard and Corliss (1951), who designated the species described by Lehman as *Syndesmis franciscana* (Lehman, 1946).

Jennings and Mettrick (1968) found a rhabdocoel in the coelomic perivisceral space and in the intestine of the urchin *Lytechinus variegatus* from Jamaican waters. Following Stunkard and Corliss (1951), Westblad (1953), who synonymised *Syndesmis antillarum* Stunkard and Corliss, 1951, with *S. franciscana*, and Hyman (1960), who reported *Syndesmis franciscana* from the gonads and intestine of *L. variegatus* from Puerto Rico, Jennings and Mettrick assigned their material to *S. franciscana*.

Studies on the nutrition of these Caribbean flatworms showed that they feed predominantly upon the ciliate Protozoa normally present in great numbers in the coelom and gut of the host urchin. This diet is also supplemented by the ingestion of small quantities of host coelomocytes. Histological and histochemical studies showed the single layered gastrodermis is apparently undifferentiated into glandular and absorptive cells. The later stages of digestion are believed to be entirely intracellular, during which the gastrodermis becomes syncytial. Of the enzymes concerned in digestion, an endopeptidase, a number of exopeptidases, acid and alkaline phosphatases, and a lipase were demonstrated in food vacuoles, although their sources were not localised.

Glycogen formed the principal food reserve in the Caribbean specimens examined, occurring in the parenchyma and constituting 15.9% of the dry weight.

A number of preserved specimens of *S. franciscana* collected from *Strongylocentrotus franciscanus* at Bodega Bay, California, were also examined, but no enzymic or biochemical determinations were made. Recently, however, it has been possible to obtain fresh specimens of *S. franciscana* from *S. purpuratus* (the second host studied by Lehman), collected at Venice, California. An examination of this new living material showed that there are morphological differences between the rhabdocoels from Jamaica and those from California. Histochemical and biochemical studies have been made on the Californian rhabdocoels, for the purposes of comparison with the Caribbean forms. Also, some ultrastructural observations have been made on the gastrodermis, to supplement histochemical attempts to localise the source of the digestive enzymes. These results compliment and support the morphological evidence that the Caribbean and Californian rhabdocoels are separate and distinct forms.

MATERIALS AND METHODS

In Californian waters, *S. franciscana* occurs in the intestine of *Strongylocentrotus franciscanus*, *S. purpuratus*, and *Lytechinus pictus* (T. Barnes, personal communication).

Two species of rhabdocoels occur in these urchins, but are easily distinguished on the basis of their colour and position within the urchin intestine. *Syndesmis franciscana* is red in colour, and is restricted to the posterior half of the intestine, with the dividing line between the two halves being taken as the point at which the digestive tube reverses direction and loops back on itself.

In the present paper most of the rhabdocoels were collected from *S. purpuratus* because this urchin was found to be easier to maintain in the laboratory than *Strongylocentrotus franciscanus* The urchins were maintained in aerated seawater tanks at 4 C, and fed on *Egregia laevigata* until examined. Collection of the worms was as previously described (Jennings and Mettrick, 1968). Living specimens were examined under phase and light microscopy.

HISTOCHEMICAL AND ULTRASTRUCTURAL METHODS

Freshly collected specimens from living *S. purpuratus* were fixed in a number of different fixatives prior to routine histological and histochemical examination for digestive enzymes by the methods summarised by Jennings and Mettrick (1968) in their study of the Caribbean form. For ultrastructural studies whole or part worms were fixed for 6 hr in 3% glutaraldehyde in veronal acetate buffer, *p*H 7.2, containing 20% sucrose, post-fixed in 1% osmic acid and 20% sucrose in veronal acetate, and embedded in epon. Sections for light microscopy were cut at 0.5–1.0 μ and stained either in basic fuchsin and methylene blue, or in Azur II. For electron microscopy grey to silver sections were obtained and stained in lead citrate, or stained twice in alcoholic uranyl acetate followed by lead citrate.

BIOCHEMICAL METHODS

As previously described (Jennings and Mettrick, 1968), batches of 200–250 rhabdocoels were collected from the urchins, washed several times in sea water to remove any debris present, and counted. The samples were taken, in three to four steps, from sea water, through sea water/distilled water, to pure, ice-cold distilled water, and finally transferred to a Virtis microhomogeniser. Samples were homogenised for 1–3 min, and the homogenate was made up to a final volume by the addition of ice-cold distilled water.

Aliquots of this homogenate, each containing at least 12 mg worm tissue (dry weight), were then taken for the following analyses.

EXTRACTION WITH TRICHLOROACETIC ACID (NONPROTEIN NITROGEN; *LYO*-GLYCOGEN).

Ice-cold trichloroacetic acid (TCA), 0.5 volume of 15%, was added to the aliquot from the original homogenate, and the suspension was allowed to stand for 10 min with occasional stirring. The sample was then centrifuged at ×3000 *g* for 10 min at 0 C. The supernatant was decanted, and the precipitate was washed twice with 1 volume of 5% TCA, and recentrifuged each time. The combined supernatants were made up to a total volume of between 10 and 15 ml by the addition of 5% TCA, and the sample was stored at 0 C.

Aliquots from this sample were removed for the estimation of nonprotein nitrogen (free amino acids) by a microkjeldahl procedure following Fleck and Munro (1964).

Further aliquots, after suitable dilution, were taken to estimate *lyo*-glycogen with anthrone reagent.

EXTRACTION OF TOTAL LIPIDS (METHOD I)

The acid-treated precipitate from the above extraction was washed with an appropriate volume of ethanol, followed by two washings of ethanol/chloroform (3:1 v/v), and further washings of ethanol/ether (3:1 v/v) and ether. All the supernatants were combined and made up to a final volume with ether. Aliquots of this solution, containing about 0.5 mg lipid/ml, were taken for estimation of total lipids following Paul's (1958) modification of Bloor's (1947) colorimetric procedure.

ALKALINE HYDROLYSIS (PROTEIN NITROGEN; *DESMO*-GLYCOGEN:RNA-P)

The precipitate remaining from method I, after lipid extraction, was dissloved in 5–10 ml 0.3 N KOH, and incubated for 1 hr at 37 C.

Aliquots from this solution were removed for estimation of protein nitrogen (tissue N) by microkjeldahl determination.

Further aliquots, after suitable dilution, were taken to estimate *desmo*-glycogen with anthrone reagent.

The remaining volume of solution was diluted with a half volume of ice-cold 0.6 N perchloroacetic acid (PCA), allowed to stand 10 min, and centrifuged for 10 min at × 9750 *g*. The precipitate was washed twice with 1 volume of 0.2 N PCA, and the supernatant and washings were transferred to a graduated cylinder. One ml of 0.6 N PCA was then added, and the final volume made up to 25 ml with distilled water to give the ribonucleic acid fraction in 0.1 N PCA.

TOTAL LIPID DETERMINATION (METHOD II)

After collecting the rhabdocoels from the sea-urchins, they were washed in sea water and counted. After as much water as possible was removed, the sample was weighed, frozen at –30 C, and then dried in a vacuum oven over Drierite for 72 hr. The sample was then reweighed and homogenized, and the lipids were extracted following the procedure of Folch et al. (1957).

OBSERVATIONS AND RESULTS

FOOD

Caribbean rhabdocoels ingest ciliate Protozoa and coelomocytes, but only fragments of these could be identified (Jennings and Mettrick, 1968); the masticating actions of the muscular pharynx effectively shredded all food during ingestion and before it reached the intestine.

In contrast, the breakdown of the food is not so complete in the Californian specimens that were examined. Gut contents were more readily identifiable, and, on occasion, sections showed apparently recently ingested ciliates lying undamaged in the intestinal lumen, with their various organelles clearly visible (Fig. 1). No significant differences in pharyngeal structure could be found, however, between Californian and Caribbean specimens, and the reasons for this difference in the degree of disintegration of the food during ingestion remains unknown.

STRUCTURE OF THE INTESTINE AND THE SITE OF DIGESTION

The intestine is a blind tube, with no pouch, whose wall, the gastrodermis, consists of a single layer of cells standing on a thin basement membrane. Throughout the length of the intestine, especially in specimens that have recently ingested large quantities of ciliates, opposing portions of the gastrodermis come together so that the entire structure becomes subdivided into a number of large vacuoles, each containing digesting food. Fixation in glutaraldehyde–osmic, with subsequent embedding in epon, gave a much clearer picture of this than was obtained previously in Caribbean specimens fixed and stained by normal histological methods. Other, smaller, vacuoles occur between the bounding membranes delimiting each cell, and also within the gastrodermal cells (Fig. 1–3). Digestion occurs within all these vacuoles and can be regarded, therefore, as both extra- and intracellular. It is affected by an endopeptidase, exopeptidases, lipase, and acid and alkaline phosphatases

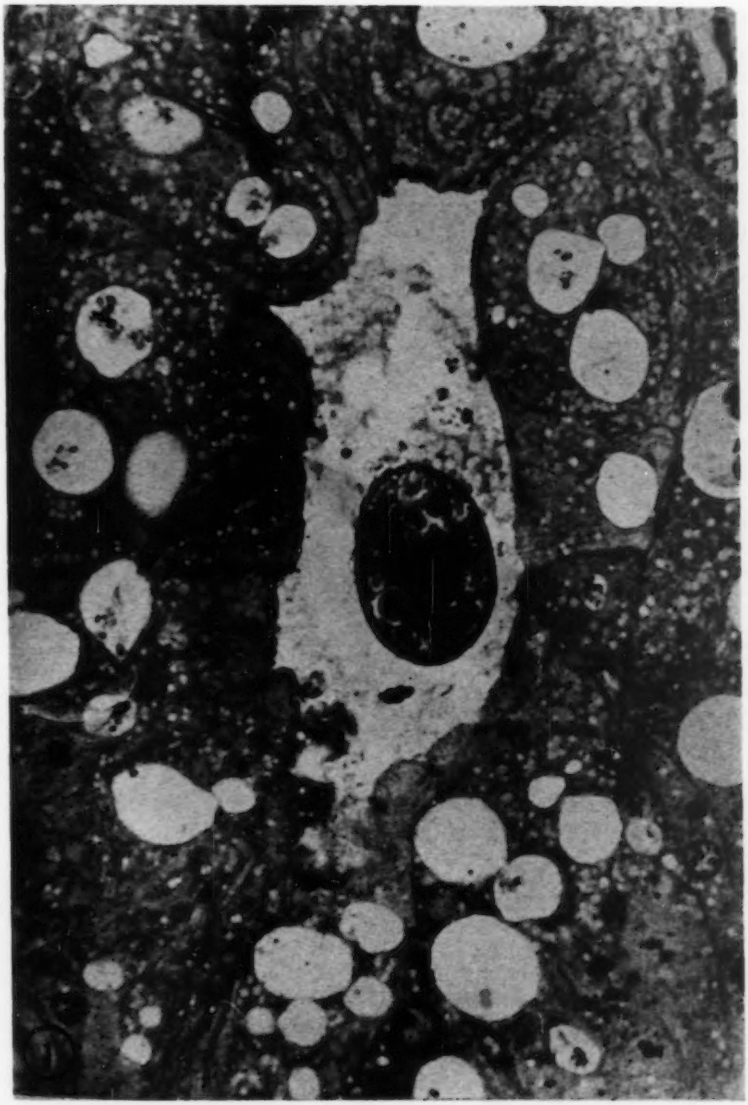

FIG. 1. Portion of the intestine of *Syndesmis franciscana* showing a transverse section of an intact protozoan, *Entodiscus borealis*, one of the most common ciliates found in the intestine of *Strongylocentrotus purpuratus*. The gastrodermal cells contain a large number of intracellular food vacuoles. 0.5-μ epon section stained with Azur II. ×480.

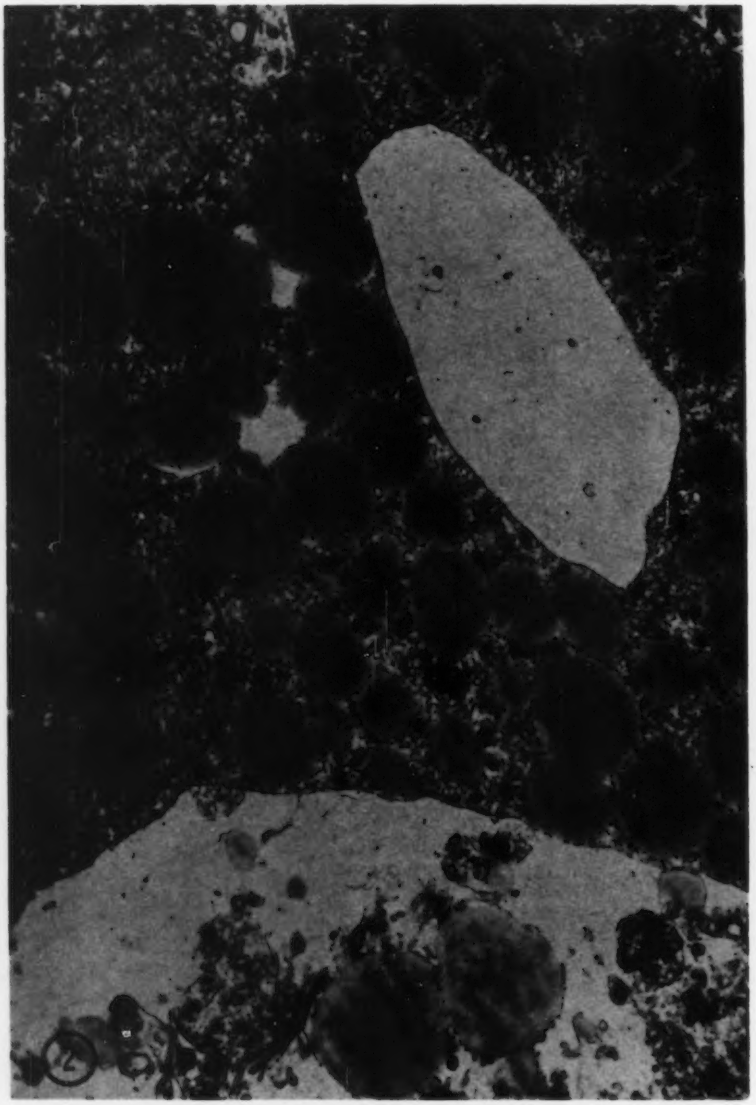

FIG. 2. Portions of two adjoining gastrodermal cells in the intestine of *Syndesmis franciscana*, showing lipid globules, and portions of two intracellular vacuoles. The vacuole at the bottom of the micrograph contains fragments of digesting ciliates and free lipid globules. Epon section, stained with lead citrate. ×3125.

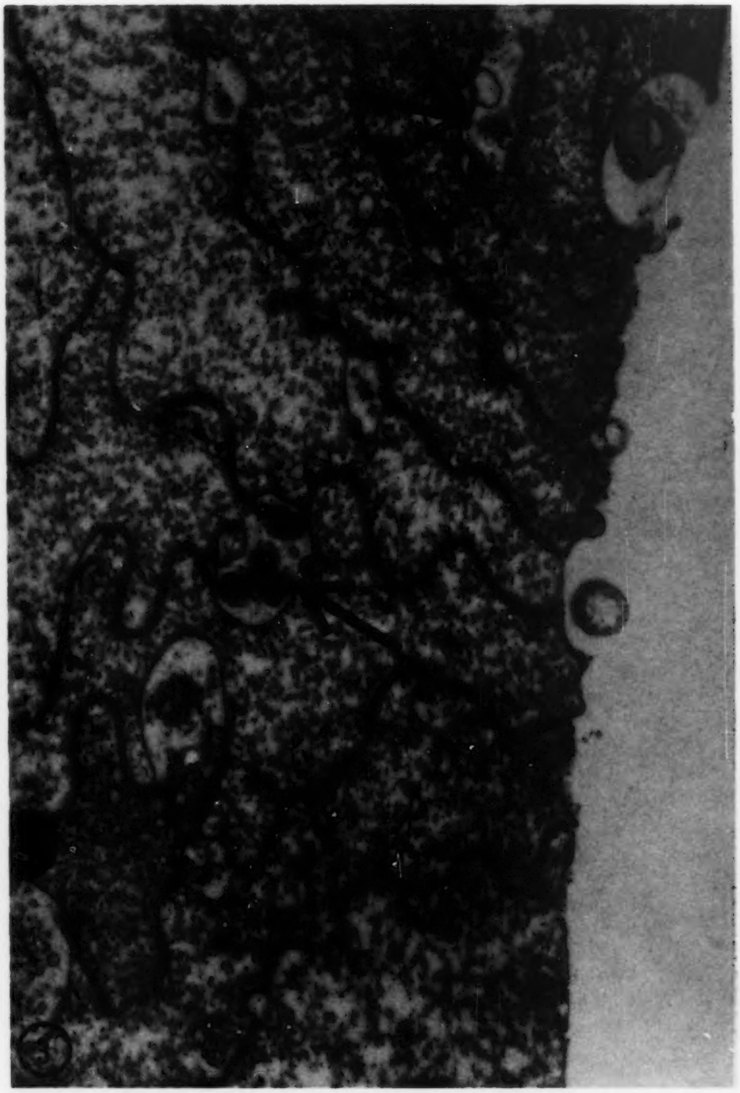

FIG. 3. Portions of a gastrodermal cell in the intestine of *Syndesmis franciscana* showing infoldings of the surface layer, enclosing several extracellular food vacuoles (marked by arrows). Epon section, stained with lead citrate. ×4500.

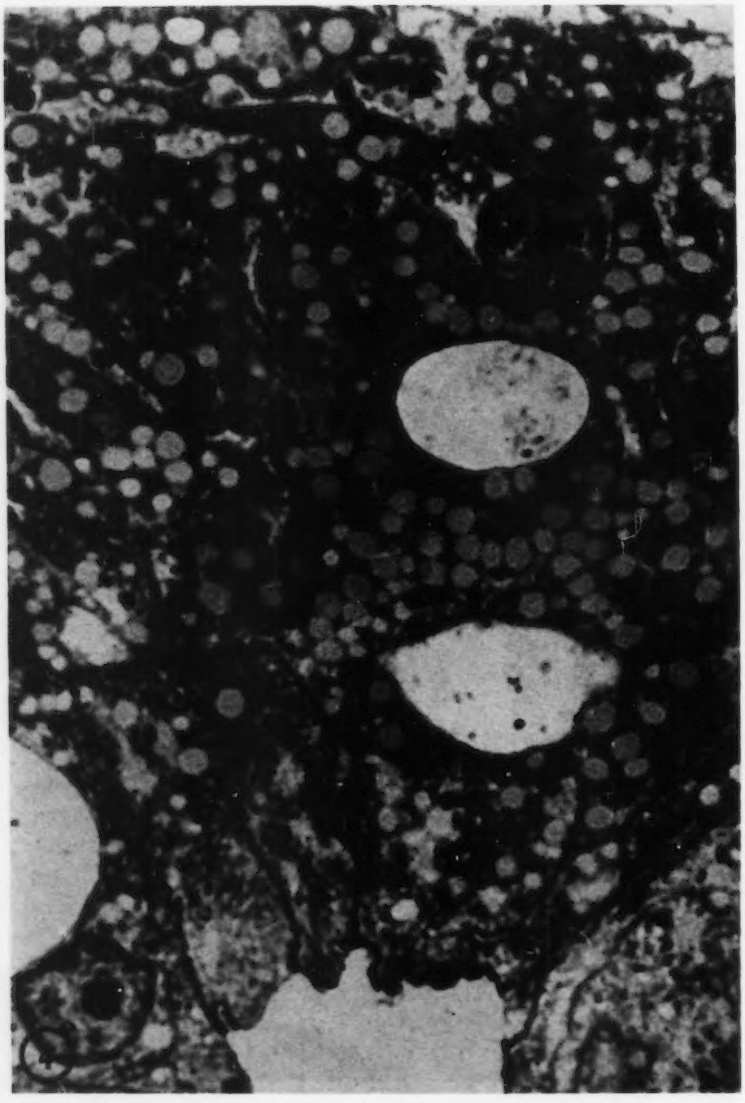

FIG. 4. Portion of the intestine of *Syndesmis franciscana* showing the gastrodermal cells loaded with lipid globules. 0.5-μ epon section stained with Azur II. ×1300.

acting in concert as described in the earlier account of Caribbean specimens by Jennings and Mettrick (1968) and secreted, presumably, by the cells surrounding or containing the vacuoles.

All the vacuoles in which digestion occurs were regarded in the Caribbean specimens as intracellular or intrasyncytial since with the fixatives then employed distinct cell boundaries could not be recognised in well-fed individuals. From the present study, through using both light and electron microscopes, it is clear that in the Californian worms the cell boundaries remain distinct and that many of the larger vacuoles appearing after ingestion of food, and containing digesting food, are strictly extracellular and represent temporarily isolated portions of intestinal lumen. This may also be true of the Caribbean specimens, but must be confirmed by electron microscopy.

Apart from this clarification of the nature of the intestinal vacuoles, the structure of the gastrodermis is essentially as described previously for the Caribbean forms, light and electron microscope studies confirming the absence of intercellular differentiations into glandular and other components. Most of the cells are 35–40 μ tall and 20–30 μ wide, but a small proportion are taller and more elongate, particularly in areas of the gastrodermis lining a large intestinal vacuole. These may well represent an early stage in the meeting of opposite portions of the gastrodermis, which will further temporarily subdivide the intestine.

With regard to cell contents, however, there is a most striking and consistent difference between the present specimens from *S. purpuratus* from California and those examined previously from *L. variegatus* in the Caribbean. Throughout the gastrodermis of the Californian specimens the cells are loaded with lipid globules 1–2.5 μ in diameter (Fig. 2, 4). The lipid nature of the globules, indicated by their yellow colouration in unstained 1-μ thick epon sections, was confirmed by their affinity for Sudan Black B when applied as a 0.5% solution in polyethylene glycol to these sections at 60 C. Oil Red O and Sudan IV in this solvent were also taken up by the globules, but to a lesser extent. Initially it was thought that the globules might represent enzymic secretions, since a loaded cell closely resembles the typical enzyme-secreting gastrodermal gland cell present in other Turbellaria (Jennings, 1962), and on occasion both intact and disintegrating cells and free globules were found extruded from the gastrodermis into the intestinal vacuoles. Other cells in situ in the gastrodermis appeared to be discharging their contained globules into the vacuoles (Fig. 2). The globules, however, consistently showed negative reactions to histochemical tests for hydrolytic enzymes and also failed to stain with Azur II or any other dyes, such as bromophenol blue, which usually stain proteinacious cell contents, and especially enzymic secretions, extremely strongly. Further, the globules do not possess a limiting membrane such as is normally present around enzymic secretions prior to their discharge (Fig. 2).

CHEMICAL COMPOSITION

The results of the biochemical analyses (Table 1) show one marked and significant difference from the data obtained for the chemical composition

TABLE 1. Chemical composition of *Syndesmis franciscana* from California.

	Per specimen (μg)	% dry wt
Protein		
Total nitrogen	8.8	8.32
Protein content	55.2	52.26
Nonprotein nitrogen	3.0	2.83
Protein nitrogen	5.8	5.48
Ratio nonprotein N:protein N	1:1.95	
RNA-P	0.4439	0.41
Carbohydrate		
Total carbohydrate	20.5	19.42
Lyo-glycogen	17.0	16.92
Desmo-glycogen	2.5	2.36
Ratio *lyo-:desmo*-glycogen	7.01:1	
Total lipids		
Method I	43.0	29.45
Method II	41.0	25.23
Dry wt	105.75	

of the Caribbean rhabdocoels by Jennings and Mettrick (1968). In those, the lipid content was extremely low and represented only 1.53% of the dry weight. In contrast the lipid content of the Californian specimens represents 29.45% of the dry weight, as determined by Paul's (1958) modification of Bloor's (1947) colorimetric procedure.

Because of the considerable disparity between the results, a second method using the technique described by Folch et al. (1957) was used for lipid determination on a fresh sample of animals. The value obtained was somewhat lower than that given by the first method (25.23% dry weight) but this is still significantly higher than the 1.53% shown by the Caribbean specimens.

With regard to the amount of glycogen present, the Californian and Caribbean specimens are remarkably similar, with glycogen forming 19% of the dry weight in the former and 18% in the latter. The values for protein content show a greater disparity, being 52% (Californian) and 67% (Caribbean).

Thus the total protein, total lipid, and total glycogen contents of specimens from California and from the Caribbean can be summarised, as percentages of dry weight, respectively as 52:29:19 and 67:2:18.

The difference in the values for protein content between the two populations appears to be greater than the difference in their total nitrogen values, from which protein content was calculated. Thus the actual difference in total nitrogen between the Californian and Caribbean specimens, expressed as a percentage of dry weight, is only 2.2% although the difference in protein content is 15%. It should, however, be remembered that although it is common practice to multiply nitrogen content by the factor of 6.25 to obtain protein content, this is, at best, only an approximation as the percentage N in different proteins does vary. Thus the difference in protein contents of the two populations cannot be regarded as being so significant as that between the respective lipid contents.

MORPHOLOGICAL COMPARISON

The most noticeable difference between the syndesmids from California and those from Jamaica, is that the former are appreciably larger, being 2.48–3.36 mm long ($\bar{x}$ = 2.78) and 1.28–1.85 mm wide ($\bar{x}$ = 1.54). These means are 50% greater than those given by Jennings and Mettrick (1968), but the size ranges are fairly close to those quoted by Lehman (1946).

Secondly, the egg capsules of the Californian species are 182–225 μ ($\bar{x}$ = 212 by 147–220 μ ($\bar{x}$ = 169). Again these means are more than 50% greater than those recorded for the Jamaican syndesmids.

In the Californian specimens the muscular base of the penis is slightly post-ovarian in position. The ratio of the length from the base of the penis to the posterior end of the body, compared to the total body length, varies between 1:3.8 and 1:8.3, with an average of 1:5.3. The ejaculatory duct is not usually coiled, although sometimes there are one or two coils near the testicular region.

In contrast the penis of the Caribbean syndesmids is very short, the muscular base being between the mid-testicular zone, and the posterior border of the testes. Thus the ratio of body length from the base of the penis compared with total body length is 1:1.5–1:1.8, with a mean of 1:1.6. The ejaculatory duct is coiled, the coils extending slightly anteriorly to the anterior margin of the testes.

DISCUSSION

A number of interesting points have emerged from this examination of *S. franciscana* from *S. purpuratus* in California, and the comparison of the findings with those reported previously by Jennings and Mettrick (1968) for *Syndesmis* specimens from the Caribbean.

Gut structure and the site and sequence of digestion would appear to be the same in both populations, but the use of different techniques has shown that a proportion of the vacuoles in which digestion occurs are truly extracellular and represent temporarily isolated portions of the intestinal lumen. The gastrodermal cells are not visibly differentiated into glandular and absorptive or phagocytic components and they remain distinct from each other during all stages of digestion and absorption.

Although gut structure is virtually identical in both populations, the Californian worms show a significant difference as regards the contents of the gastrodermal cells, which are loaded with lipid globules. Biochemical assay shows that a substantial proportion of the dry weight is in fact lipid; in contrast, the gut cells of Caribbean specimens contain very little lipid and the total lipid proportion of dry weight is less than 2%. The physiological significance of this difference in lipid content is not known, but it may be linked more to the fact that the two *Syndesmis* populations originated from two different host species rather than to some other factor related to their geographical locations. The role of the large quantity of lipid in the Californian *S. franciscana* similarly is unknown; the globules may represent stored food materials, or they may simply be intermediate stages between absorption of the products of digestion and onward passage of these to the rest of the body. The lipid globules discharged into the gut lumen and temporary intestinal vacuoles in apparently both "merocrine" and "holocrine" fashion may well be artefacts resulting from the convulsive movements of the flatworms during fixation. Another possibility, however, is that the lipid is being actively "excreted," perhaps as a consequence of a high lipid content in the ciliate Protozoa that form the bulk of the diet.

The glycogen content of both Caribbean and Californian umagillids is remarkably similar, and is high relative to the values found in free-living helminths. High glycogen content, however, is characteristic of endoparasitic helminths, and Jennings and Mettrick (1968) suggested, therefore, that the basal metabolism of *S. franciscana* is probably more akin to that of trematodes and cestodes than to other turbellarians. This view is further supported by the present work, in which the total glycogen content of 19.43% was found to result from 16.92% as soluble *lyo*-glycogen and 2.36% as bound *desmo*-glycogen. This pattern appears to be characteristic of glycogen disposition in endoparasitic helminths. Dougherty and Taylor (1956), using TCA as an extractant, have shown than about 80% of the glycogen content in adult *Hymenolepis diminuta* is in the soluble *lyo*-form. In contrast, Hopkins (1960) showed that in the larvae of *Hydatigera taeniaeformis* over 60% of the glycogen was of the bound *desmo*-type. Hopkins suggested that since these larvae live in a homeostatically controlled environment, fluctuations in exogenous glycogen will be small. In contrast, the adult cestode, living in an environment where exogenous carbohydrate supplies vary greatly depending upon the time and nature of the host's previous meal, will require most of its glycogen reserves to be labile to supplement exogenous sources as required. The situation in *S. franciscana* appears analogous with that of adult *H. diminuta* and it is, therefore, particularly interesting that about 87% of the total glycogen content, itself of a level comparable with that found in endoparasitic rather than free-living flatworms, should be in the soluble *lyo*-form.

Turning to the morphology of the Californian syndesmids, Lehman (1946) unfortunately did not give the size of the egg capsules in his original description of *Syndisyrinx franciscanus*. He states that the length of his specimens were between 2 and 3 mm, although the worms shown in fig. 1 and 2 of his paper (Lehman, 1946, p. 298, 300) are both over 4 mm long, based on the scales provided.

Lehman also describes the base of the penis at "about one-third of the distance from the posterior end of the body." In fig. 2 (p. 300) the base of the penis is behind the common ovarian trunks, and level with the anterior border of the cement gland. This arrangement agrees very well with that found in all the Californian material that we have examined, although in our specimens the actual position of the base of the penis varies considerably in relation to the total length of the specimen, but not in its relation to the ovary and cement gland.

Westblad (1953), who commented that many years previously he had received some preserved turbellarians from the body cavity of *Diadema* (*Centrechinus*) *antillarum*, considered that Powers' material (1936) from the same host was identical with *S. franciscana*, and concurred with Marcus (1949), who had synonymised *Syndesmis* with *Syndisryrinx*.

Hyman (1960) agreed with Westblad (1953) that the umagillid from Florida described and figured by Powers (1936) was *Syndesmis franciscana*, and pointed out that the specific name *antillarum*, suggested by Stunkard and Corliss (1951) for Powers' material, was a synonym of *franciscana*. Stunkard and Corliss (1951) described the muscular base of the penis in *S. antillarum* as being near the middle of the body, and showed that the ejaculatory duct was coiled. More significantly their figure of *S. antillarum* based on that of Powers, shows that the base of the penis is slightly post-testicular. This is in marked contrast to the adjacent figure of *S. franciscana* (Stunkard and Corliss, 1951, pl. 1, fig. 4, 5, p. 321) in which the base of the penis is nearly post-ovarian. On the basis of our examination of about 100 specimens, both living and preserved, these differences between the Californian and Caribbean syndesmids appear to be constant, and significant.

In the type species of this genus, *Syndesmis echinorum* François, 1886, best figured by Russo, 1895, the base of the penis is post-ovarian, and the ejaculatory duct is considerably coiled. Similarly in *S. franciscana* and *Syndesmis punicea*, Hickman, 1956, the base of the penis is at the level of the ovary or even post-ovarian. (The relations of the seminal receptable and seminal bursa in these two species are also very similar and they are probably synonymous.) In addition to the position of the base of the penis, the length of the uterus may be used to separate the Caribbean syndesmids from *Syndesmis glandulosa* Hyman, 1960, and *Syndesmis evelinae* Marcus, 1968.

Thus the syndesmids described from Jamaica in our previous paper (Jennings and Mettrick, 1968) differ from all the *Sydesmis* species at present assigned to the genus (Marcus, 1968). The Jamaican species appears, as far as the original description goes, to be identical with *S. antillarum* Stunkard and Corliss, 1951. In passing, it should be noted that Powers' original specimens were collected from *Diadema antillarum* examined at the Tortugas Laboratory, off the Florida Coast. We did not find any Syndesmids in *D. antillarum* from Jamaican waters.

We therefore propose that *S. antillarum* be recognised as a valid species, based on Powers' original description, the description given by Jennings and Mettrick (1968), and the further details recorded above. It is unfortunate that the specific name previously selected is now so inappropriate!

Although the differences in nutritional physiology, principally in the amount of stored lipid, are significant, they could be explained as a simple reflection of the fact that these rhabdocoel flatworms are entocommensal in different host species, harbouring different species of Protozoa, so that the worms have different diets, with consequent variation in the composition and relative proportions of the dietary constituents.

However, when coupled with the morphological differences between the Caribbean and Californian syndesmids, it is clear that the Californian and Caribbean worms are two separate species.

SUMMARY

1. Histochemical, biochemical, and ultrastructural studies have been made on the nutritional physiology of *S. franciscana*, a rhabdocoel entocommensal in the coelom and intestine of the sea-urchin *S. purpuratus* from California, and the findings compared with earlier investigations on specimens of a *Syndesmis* species entocommensal in the urchin *L. variegatus* in the Caribbean.

2. The Jamaican syndesmid is now considered to be identical with *S. antillarum* Stunkard and Corliss, 1951. The present synonymy of *S. antillarum* with *S. franciscana* is shown to be unjustified on both morphological and physiological grounds.

3. The structure of the intestine of *S. franciscana* is redescribed. Many of the large vacuoles consistently present in recently fed individuals are shown to be entirely extracellular, and to represent temporarily isolated regions of the intestinal lumen in which digestion is occurring.

4. Digestion is both extra- and intracellular, and an endopeptidase, exopeptidases, phosphatases, and a lipase have been demonstrated in the food vacuoles.

5. The gastrodermal cells in *S. franciscana* are loaded with lipid, and this substance forms over 25% of the dry weight as compared with less than 2% in Caribbean syndesmids.

6. The glycogen content in *S. franciscana* is over 19% of the worm dry weight. This value is very similar to that previously obtained for the Caribbean species. The bulk of the glycogen is shown to be present in the soluble *lyo*-form, as in adult endoparasitic helminths.

7. It is suggested that differences in the nutritional physiology, principally in the amount of stored lipid, in the two species of *Syndesmis* could be due to their occurrence in different host species and may represent a stage in the physiological divergence of the two forms related to host specificity.

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Some Effects of Shape and Structure of Fish on Selectivity of Gillnets[1]

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McCombie, A. M., and A. H. Berst. 1969. Some effects of shape and structure of fish on selectivity of gillnets. J. Fish. Res. Bd. Canada 26: 2681–2689.

Selectivity curves for three species of fish taken in South Bay, Manitoulin Island, and in Georgian Bay during 1954–59 with gangs of nylon gillnets differed with respect to mode, skew, and kurtosis. A graphic method was developed to describe one aspect of body shape, namely the relation between girth and distance from the snout, which would affect selectivity. Surprisingly, the shapes of mature (but not gravid) and immature perch and suckers of both sexes could be described by the same curve. Hence, differences in body shape did not account for differences in the selectivity of these species. In contrast, the taper for gravid female perch was markedly different from that for nongravid, as might be expected. Catches in which the positions where caught were carefully identified showed that most of the fish were caught either on the opercle or near the position of maximum girth. That the fish could be caught at different positions accounted for most of the skew in the selectivity curves based on maximum girth. Selectivity curves plotted on the basis of girth where caught, rather than on maximum girth or length, show that efficiency of capture was maximal when the girth of the fish was 1.0–1.2 times as great as the perimeter of the mesh. Efficiency was negligible at girth/perimeter ratios smaller than 0.8 or 0.9 and it declined rapidly at ratios greater than 1.2. However, a few fish were taken at ratios up to 1.5 or 1.6.

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La façon dont les filets maillants sélectionnent des poissons de tailles différentes peut être décrite par une courbe qui ressemble à une distribution normale ayant plus ou moins de biais positif. Les auteurs tracèrent de telles courbes pour les perchaudes, catostomes noirs communs, ménomminis d'Amérique et hareng de lac pris dans la Baie du Sud et La Baie Georgienne (lac Huron) de 1954 à 1959 au moyen d'une série de filets en nylon de mailles variant de 38 mm à 114 mm. Ces courbes diffèrent quant au mode, au biais et au kurtose. Une méthode graphique fut developpée pour décrire une aspect de la forme d'un poisson qui peut affecter la sélection, à savoir le rapport de la sangle avec l'éloignement du bout du mouseau. Étonnament l'on découvrit que les formes des perchaudes et des catostomes, tant mûrs que pas mûrs, qui paraissent si différentes à l'oeil, peuvent être décrites par une seule courbe. D'autre part, la forme d'une femelle perchaude gravide diffère complètement de celle d'une perchaude non gravide, comme l'on s'imaginerait. Les expériences dans lesquelles l'on identifia soigneusement la position d'enchevêtrement démontrèrent que le plupart des poissons furent pris soit à l'opercule soit à la position de la plus grande sangle. Que les poissons furent pris en différentes positions expliqua beaucoup du biais dans les courbes de sélection fondées sur la plus grande sangle. Les

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courbes fondées sur la sangle à la position d'enchevêtrement démontrèrent que le poisson fut pris avec le plus d'efficacité quand cette sangle fut 1.0 à 1.2 fois plus grande que le périmètre de la maille. L'efficacité fut négligeable quand le rapport plus grande sangle/périmètre de la maille égala 0.8 à 0.9 et elle diminua rapidement quand le rapport dépassa 1.2. Cependant quelques poissons furent attrapés aux rapports jusqu'à 1.5 ou 1.6.

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INTRODUCTION

DURING THE PAST DECADE fisheries biologists have become increasingly concerned with describing the selectivity of gillnets and similar gear for fish of different sizes (Graham and Mann, 1959; Olsen, 1959; Garrod, 1961; Holt, 1963; Kipling, 1963; Pycha, 1963; Ishida, 1962a, b, 1963, 1964; Regier and Robson, 1966; Olver, MS, 1966; Trent and Hasler, 1968; Kitahara, 1968; and Lagler, 1968). The statisticians wish to know to what extent population statistics estimated from gillnet catches may be biased by selectivity. Management would like to predict what effect any proposed change in mesh regulations might have on the size composition of the catch.

On analysing catches of the lake whitefish (*Coregonus clupeaformis* (Mitchill)) taken in experimental gangs of gillnets of different meshes, McCombie and Fry (1960) concluded that the selectivity for this species could be described by a single curve in which frequency of capture was plotted against the ratio maximum girth of fish/mesh perimeter. An approximate fit to the normal distribution was obtained by transforming the abscissa to logarithms or by using the method of moments (McCombie, 1961). Subsequently, Berst (MS, 1960, 1961) obtained similar selectivity curves for the yellow perch (*Perca flavescens* (Mitchill)), white sucker (*Catostomus commersoni* (Lacépède)), round whitefish (*Prosopium cylindraceum* (Pallas)), cisco (*Coregonus artedii* (LeSueur)), and one or more unidentified coregonids of the chub group.

The latter study showed that there was need for more detailed investigations into the mechanics of capture. Although the selectivity curves for all species investigated resembled the normal distribution, the fit varied considerably from one species to another. There were also differences in skew and the curve for the yellow perch was markedly leptokurtic.

Accordingly, the present work was undertaken to investigate the effects of interspecific differences in body shape and of protruding anatomical structures on gillnet selectivity. That these factors might be significant had been suggested by Moyle (1949) and by Gulland and Harding (1961), but they had not received the attention they deserved. The results of the experiments described here show that these effects do indeed account for some of the differences in the shapes of selectivity curves and should be taken into account in future mathematical formulations.

To date, most net selectivity curves have been based on the length of the fish for the obvious reason that this dimension is most easily and most often measured. On the other hand, Margetts (1954) and Messtorff (MS, 1958) investigated the relation between selectivity and girth for certain marine species. In the present study, girth was chosen in preference to length because the prime purpose was to investigate the relation between selectivity and the fit of the fish to the mesh. A ratio based on the relation between fish girth and mesh perimeter would seem to be the most direct measure of fit.

METHODS

The catches of yellow perch, white suckers, round whitefish, and ciscoes on which this study is based were taken from South Bay, Manitoulin Island, and from Georgian Bay during the years 1954–59. These catches were part of the routine monitoring of fish stocks conducted from the Ontario Department of Lands and Forests, Fisheries Research Station, at South Baymouth. With one exception, noted below, they were taken with gangs of nylon gillnets having the following dimensions:

Mesh size (*cm*) (stretched knot to knot)	3.8	4.4	5.1	5.7	6.3	7.0	7.6	8.9	10.2	11.4
Thread size	$\frac{140}{2}$	$\frac{140}{2}$	$\frac{140}{2}$	$\frac{140}{2}$	$\frac{140}{2}$	$\frac{140}{2}$	$\frac{210}{3}$	$\frac{210}{3}$	69	69
Meshes deep	48	40	36	32	30	26	24	24	24	18

In 1954, 1955, and 1956 each net was 46 m (50 yards) long but in 1957 the length was increased to 137 m (150 yards). All nets were hung on the half in ascending order of mesh size.

In the routine for sampling catches the total weight of each species taken in each mesh size was recorded first. Then samples were taken from each mesh, and the species, sex, state of maturity, fork length, and weight of the specimens were recorded.

In 1958 and 1959 maximum girth measurements were made on 100 or more specimens of each species to permit conversion from fork length to maximum girth. These girths were measured with the apparatus described by Berst and McCrimmon (1961), an improved version of which has since been described by Wydoski and Wolfert (1968). It was found that over the range of fish sizes encountered, the relation between maximum girth and fork length could be described by a linear regression (McCombie and Fry, 1960; Berst, MS, 1960).

For the studies of body shape seven or eight positions were selected on the fish body (Fig. 1). and the following additional measurements made: (1) the girth at each position, and (2) the

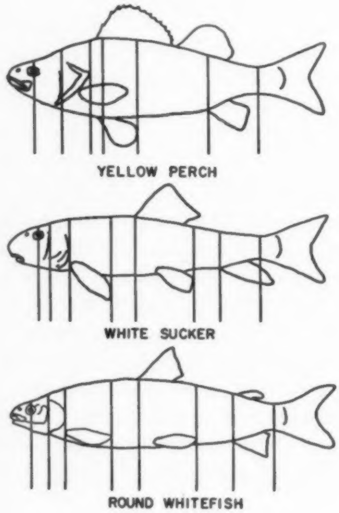

FIG. 1. Positions (indicated by the vertical lines) at which girth measurements were made for studies of body shape.

distance from the snout to each position. Samples contained immature and mature fish of both sexes and several size classes.

The only catches that were not taken in gillnets were poundnet catches of female perch, which were both mature and ripe. Data from these specimens were used to plot the body shape curve for this group. Gillnets were not fished in June, when this species was spawning, and hence it was not possible to derive the corresponding selectivity curve for gravid females.

To find out at what point or points on its body a fish might be caught and what role protruding structures such as the premaxilla, preopercle, or opercle might play in capture, the following procedure was developed. As experimental gangs of gillnets were brought aboard and cleared an attempt was made to identify, as closely as possible, the point at which the loop (or loops) of mesh entangled each fish. The point was marked with a knife incision so that it could be recognized later when the catch was landed.

OBSERVATIONS AND DISCUSSION

Figure 2 illustrates how the selectivity curves, on the basis of maximum girth of fish, for three of the species taken from South Bay differ with respect to their ranges, modes, and skew. The skew, as measured by the third moment, is 0.59, 3.83, and 0.16 for the perch, sucker, and round whitefish, respectively, and the respective modal girth/perimeter ratios are 1.18, 1.16, and 1.35. The corresponding means are 1.22, 1.25 and 1.41.

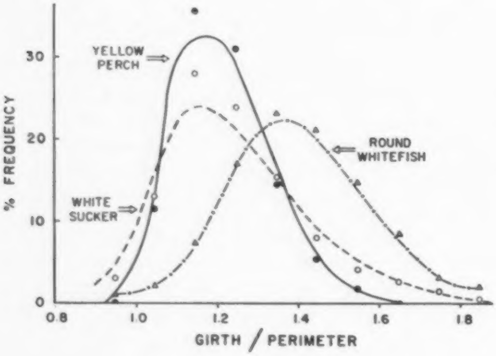

FIG. 2. Gillnet selectivity curves, on basis of maximum girth of fish in relation to perimeter of mesh, for three species caught in South Bay. The curves were fitted by the method of moments.

In contrast the selectivities for the same species caught in two different waters are almost identical (Fig. 3). The total numbers of perch, suckers, and ciscoes sampled from South Bay and used to calculate the data points in Fig. 3 were 12,358, 1627, and 1211, respectively. The corresponding numbers from Georgian Bay were 3676, 911, and 1883. Hence the small differences between the two sets of points may be due to differences in sample size.

It is also evident that the method of moments provides an approximate fit only and that there is room for improvement in the mathematical model (Fig. 2). In particular, the observed data for suckers and perch are appreciably higher than the calculated values in the neighbourhood of the mode.

Although the body shapes of the species considered here are visibly different, it is desirable in the present context to have some graphic means of

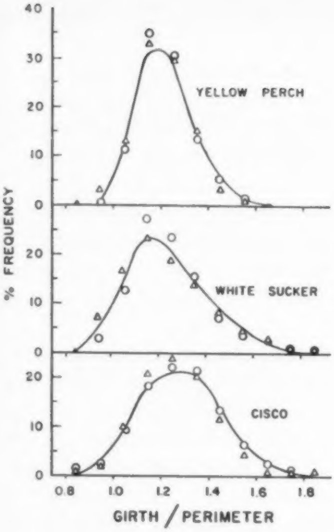

FIG. 3. Gillnet selectivities, on basis of maximum girth of fish in relation to perimeter of mesh, for three species taken in South Bay (○ and fitted curves) and for the same species taken in Georgian Bay (△). Curves fitted by method of moments.

describing these shapes as they would affect gillnet selectivity. Such a "net's eye view" (Fig. 4) was derived by plotting the girth at each predetermined position (Fig. 1) against the distance from the snout to the respective position. To eliminate the effect of size differences between specimens and allow the data to be pooled, the distances from the snout and the girths were transformed to percentages of the fork length. The datum points in the figure

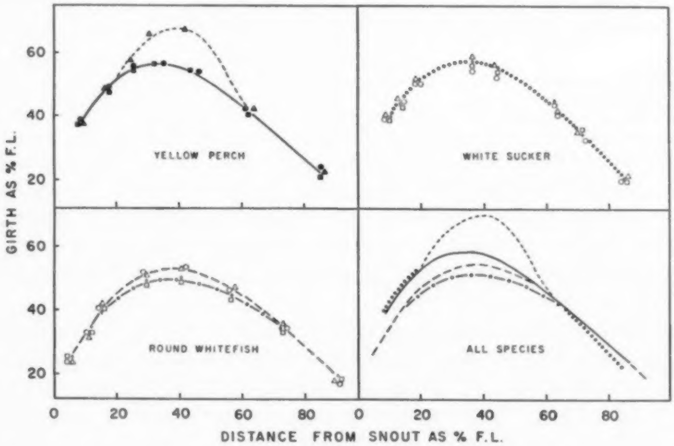

FIG. 4. Curves illustrating differences in body shapes for different species and states of maturity. +, immature male; △, mature male; ●, ripe male; □, immature female; ○, mature female; ▲, gravid female; ■, spent female.

represent these percentage data averaged according to species, sex, and state of maturity.

Because the samples of fish for body shape measurements were taken during the summer, only the yellow perch, a late spring spawner, showed appreciable sexual dimorphism (Fig. 4). Gravid female perch were much stouter than spent females or ripe males, a difference that was obvious even before the measurements were made.

In contrast, the data for ripe male and spent female perch could be fitted with a single curve. Surprisingly, the same curve could also be fitted to the data for mature (but not gravid) and immature white suckers of both sexes. Hence these two fishes, which appear quite different in shape to the human eye, are the same to the net.

Although the selectivity curve for gravid perch was not obtained in the present investigation, it seems likely from the marked difference in body shape shown here that it would differ from that for males and nongravid females. In this connection it should be noted that effects of sexual dimorphism and condition factor on the selectivity of herring drift nets were reported by Tester (1935) and by Farran (1936).

The bodies of mature male and female round whitefish are measurably stouter than those of immature specimens. At the same time the anterior and middle portions of the bodies of both mature and immature round whitefish are notably slimmer than the same portions of the perch and sucker.

From the catches in which the positions of entanglement were marked, it was found that perch and suckers were enmeshed by the preopercle, opercle, and body, whereas the round whitefish were caught by the opercle and body only. Fish caught by the body were, with few exceptions, entangled at the point of maximum girth. The percentages of fish caught at the different positions were:

	Preopercle	Opercle	Maximum girth
Yellow perch	10	60	30
White sucker	7	62	30
Round whitefish	Nil	73	26

Since the premaxilla is closely attached to the skull in the three species dealt with here, none of the specimens was caught by this structure. These results are similar to those of Farran (1936) in Irish coastal herring fisheries. Some herring were taken by the maxilla, preopercle, and opercle but most were caught between the maximum girth and the posterior margin of the opercle (see also Holt, 1963, p. 112).

The fact that the fish can be caught at different body positions contributes in varying degrees to the skew in selectivity curves based on maximum girth (Fig. 5 left-hand panel,). There is little skew in the selectivity curve for the nongravid yellow perch because the posterior end of the opercle and the position of maximum girth coincide in this species (Fig. 1). Hence the modal values of maximum girth/mesh perimeter are identical. Rather, the component curves for perch caught by the opercle and by the body reinforce each other

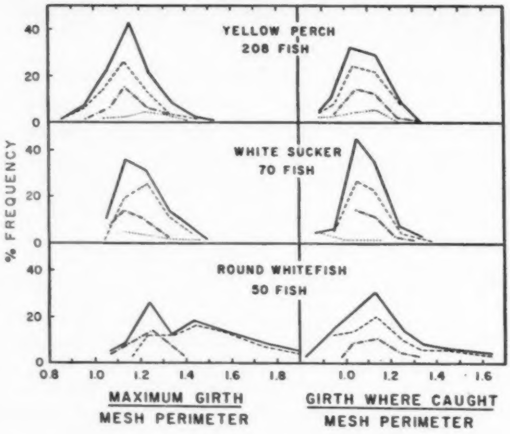

FIG. 5. Selectivity curves for the experiments in which position of and girth at the point of entanglement were recorded. —, total catch; – – –, caught on opercle; – . –, caught on body; . . . caught on preopercle.

to produce leptokurtosis. In contrast there is a marked positive skew in the selectivity curve for white suckers, a species in which the girth at the posterior end of the opercle is smaller than, and at some distance from the position of, maximum girth. The modal values of maximum girth/mesh perimeter for suckers caught by the opercle and body are 1.24 and 1.14 respectively.

That the selectivity curve, on the basis of girth of fish where caught, for round whitefish is more skewed and has a lower mode and a wider range than the curve for the white sucker (Fig. 5, right-hand panel) may be due to the fact that the forepart of the body is somewhat more tapered in the round whitefish (Fig. 1). The body of the round whitefish is fusiform whereas that of the white sucker is cylindrical. The modal values for maximum girth/mesh perimeter for round whitefish caught by the opercle and body are 1.44 and 1.25, respectively.

In perch and white sucker some skew may be introduced into the descending arm of the selectivity curve by fish caught on the preopercle.

As a result of basing the selectivity curves for the three species considered here on the girth at the position of entanglement (Fig. 5, right-hand panel), rather than on maximum girth, the modes for fish caught at different positions are brought to a common value, which is closer to unity than the modes for curves based on maximum girth. Hence it is evident that for most of the fish captured the girth at the position of capture is equal to, or slightly greater than, the perimeter of the mesh.

In contrast very few fish were captured when the girth/perimeter ratio was less than 0.8 or 0.9 (Fig. 5, right-hand panel). The authors suggest that, when a fish has a maximum girth 10 or 20% smaller than the perimeter of the mesh, it can probably swim through the net with as little hindrance as it would through a stand of rooted aquatics.

For the species studied, as the ratio of girth where caught to perimeter of mesh exceeds 1.2, the efficiency of capture declines rapidly. No perch or suckers were taken at ratios greater than 1.5 and less than 5% of the round whitefish were caught at the ratio of 1.6.

That a fish can be caught when its girth is 50–60% greater than the perimeter of the mesh may be surprising on first consideration. However, the elastic properties of nylon thread and the fact that a mesh can compress the body of the fish should be borne in mind. In the latter regard it may be significant that round whitefish, which have the most delicate scales of the three species considered, were taken at girth/perimeter ratios up to 1.6.

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The authors are much indebted to Messrs W. J. Christie and K. H. Loftus for reviving interest in the effects of fish shapes on the selectivity of gillnets. The data on which this paper is based were collected with the help and encouragement of Dr F. E. J. Fry and the late J. C. Budd. The assistance of the staff of the South Bay Fisheries Research Station of the Ontario Department of Lands and Forests is gratefully acknowledged. We are also grateful to M.D. Meunier for editing the French abstract.

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Records of Mesopelagic and Other Fishes from the Canadian Atlantic with Notes on their Distribution

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The following 35 species are recorded from the Canadian Atlantic: *Bathylagus euryops*, *Cyclothone* spp., *Maurolicus muelleri*, *Argyropelecus lychnus lychnus*, *Chauliodus sloani*, *Stomias boa ferox*, *Chlorophthalmus agassizi*, *Benthosema glaciale*, *Ceratoscopelus maderensis*, *Diaphus dumerili*, *D. termophilus*, *Hierops arctica*, *Hygophum hygomi*, *H. macrochir*, *Lampanyctus alatus*, *L. festivus*, *L. pusillus*, *Lobianchia dofleini*, *Myctophum punctatum*, *Notolychnus valdiviae*, *Notoscopelus krøyeri*, *N. resplendens*, *Symbolophorus veranyi*, *Notolepis rissoi krøyeri*, *Nemichthys scolopaceus*, *Nessorhamphus ingolfianus*, *Polymixia lowei*, *Trachurus trachurus*, *Decapterus macarellus*, *Stenotomus chrysops*, *Lycodes esmarki*, *Centrolophus niger*, *Psenes maculatus*, *Helicolenus dactylopterus*, and *Icelus bicornis*. The composition of the mesopelagic fauna of the Slope Water region and the vertical migrations of its components are discussed.

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INTRODUCTION

During cruises by vessels of the Fisheries Research Board of Canada on the Nova Scotia banks and in the Gulf of St. Lawrence from April 1967 to February 1969, a number of fish species were caught that were previously unrecorded or seldom recorded from the area. The majority of these were mesopelagic species captured in an Isaacs-Kidd midwater trawl over bottom depths between 100 and 750 fath. A recent review of the fishes of the Canadian Atlantic (Leim and Scott, 1966) indicated that this faunal component is virtually uninvestigated in the Canadian region. Recent suggestions that mesopelagic fishes are a resource suitable for commercial exploitation (e.g., Gulland, MS, 1968) make a knowledge of the species composition, distribution, and abundance of particular importance.

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In the following list of species, data are given on depth of capture. When specimens were taken by otter trawl the depth at which the gear was fishing, and hence the depth of capture, could be accurately recorded to the nearest fathom by echo sounder, although the possibility exists of specimens being taken at intermediate depths when hauling in the trawl. However, the Isaacs-Kidd midwater trawls used had no depth recording, or opening and closing devices, and the depth fished is an estimate calculated from length of warp out and wire angle. The lack of closing mechanisms on the nets would allow contamination of deep tows with organisms living at lesser depths during setting and hauling in. Furthermore, although the nets were thoroughly washed down after each tow, contamination from previous tows by fish stuck in the meshes of the net certainly occurred on some occasions. Thus, the depth records of specimens taken by Isaacs-Kidd nets must be considered in the light of these sources of error.

For mesopelagic species, time of capture is also given, since depth distribution is closely related to time of day for species that undergo diurnal vertical migrations.

References to previous distributional records from the Canadian region are cited only if they are not included in the review by Leim and Scott (1966). Fish lengths are given as standard lengths, i.e., excluding caudal fin. Almost all the specimens recorded here are deposited in the Royal Ontario Museum, Accession No. 1361, 1428, 1465, 1500.

The localities mentioned in the text are shown in Fig. 1.

LIST OF SPECIES

ORDER Isospondyli

Family BATHYLAGIDAE

Bathylagus euryops Goode and Bean 1895, goitre blacksmelt

One specimen 9.2 cm, at 110 fath, 2200 hr, July 1968, by Isaacs-Kidd net fishing south of La Have Bank. Bottom depth 380–740 fath.

Family GONOSTOMATIDAE

Cyclothone spp.

Twenty-three specimens, 1.7–4.1 cm, between 115 and 260 fath, 0800–1800 hr, July 1967, by Isaacs-Kidd net fishing south of La Have Bank. Bottom depth 220–400 fath.

Several of these specimens can be allocated to *Cyclothone microdon* (Günther, 1878) but the others cannot be confidently identified at this time. At least two species are present in the collection.

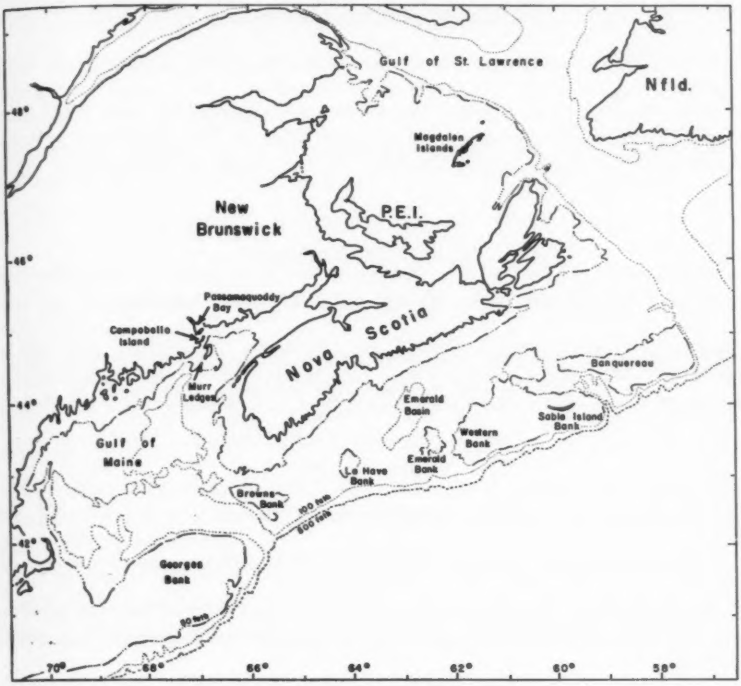
Gulf of St. Lawrence
Nfld.
Magdalen Islands
48°
New Brunswick
P.E.I.
46°
Passamaquoddy Bay
Campobello Island
Nova Scotia
Murr Ledges
Banquereau
44°
Emerald Basin
Sable Island Bank
Western Bank
Gulf of Maine
Emerald Bank
La Have Bank
Browns Bank
100 fath
500 fath
42°
Georges Bank
50 fath
70°
68°
66°
64°
62°
60°
58°

FIG. 1. Localities mentioned in text.

Maurolicus muelleri (Gmelin 1788), Müller's pearlsides

Twenty-six specimens, 1.0–4.3 cm. Twenty taken between the surface and 220 fath, May–July 1967, July 1968, by Isaacs-Kidd net over depths of 85–740 fath. Areas of capture ranged from south of La Have Bank to southeast of Sable Island Bank including one specimen from the Emerald Basin. Six taken by otter trawl between 30 and 220 fath, four being from southeastern La Have Bank in July 1967, one from southeastern Browns Bank in July 1968, and one from Passamaquoddy Bay in November 1968.

In a recent review of the distribution of this species in the northwestern Atlantic, Steele (1967) lists 56 records, 17 of which are from Canadian waters between Banquereau and Campobello Island. It is, therefore, quite common in southern Canadian waters.

Family STERNOPTYCHIDAE

Argyropelecus lychnus lychnus Garman 1899

One specimen, 4.8 cm, at 130 fath, 1400 hr, July 1967, by Isaacs-Kidd net, southeast of La Have Bank.

Family CHAULIODONTIDAE

Chauliodus sloani Bloch and Schneider 1801, viperfish

Seven specimens, 4.1 (approx)–17.0 cm, between 30 and 260 fath, by Isaacs-Kidd net, six from south of La Have Bank in July 1967 and July 1968, one from south of Sable Island Bank in October 1968. Bottom depth 255–695 fath.

Family STOMIATIDAE

Stomias boa ferox Reinhardt 1842, boa dragonfish

Sixty-eight specimens, 7.6–23.5 cm, between the surface and 260 fath in July 1967, July and October 1968, and January 1969, by Isaacs-Kidd net fishing south of La Have and Sable Island banks. Bottom depths 225–740 fath.

ORDER Iniomi

Family CHLOROPHTHALMIDAE

Chlorophthalmus agassizi Bonaparte 1840, shortnose greeneye

Five specimens, 5.0–10.5 cm, trawled between 70 and 90 fath southwest of Sable Island Bank (43°18′N, 60°58′W) in January 1969. Bottom temperature 5.1–5.2 C.

These records confirm the conclusions of Halliday (1969) that *C. agassizi* is a year-round resident on the western Nova Scotia banks and extend the species range 140 miles to the east.

Family MYCTOPHIDAE

Benthosema glaciale (Reinhardt 1837), glacier lanternfish

4350 specimens taken by Isaacs-Kidd net from southwest of La Have Bank to southeast of Sable Island Bank. This is the most abundant member of the mesopelagic fauna. Its distribution will be treated in detail in a later publication.

Ceratoscopelus maderensis (Lowe 1839), horned lanternfish

Seventy-six specimens, 1.8–7.0 cm, between 10 and 240 fath in May and July 1967, February, July, and October 1968, and January 1969. Seventy-five were collected by Isaacs-Kidd net over bottom depths of 160–740 fath, one by otter trawl in 200 fath. All were taken from southwest of La Have Bank to southeast of Sable Island Bank. A further 31 specimens were found in the stomachs of silver hake (*Merluccius bilinearis*) caught in 195 fath between Browns and La Have banks in February 1969.

Diaphus dumerili (Bleeker 1856)

Four specimens, 2.6–3.6 cm, between the surface and 56 fath, 1800–0200 hr, July and October 1968, by Isaacs-Kidd net south of La Have and Sable Island banks. Bottom depth 400–715 fath.

Nafpaktitis (1968) records 42 specimens south of Nova Scotia in the Slope Water region.

Diaphus termophilus Tåning 1928

One specimen, 3.7 cm, at 30 fath, 0100 hr, July 1968, by Isaacs-Kidd net south of La Have Bank. Bottom depth 675–695 fath. Previously unrecorded from the Canadian region.

(Both this species and *D. dumerili* were identified by using Nafpaktitis' (1968) key to the North Atlantic members of the genus.)

Hierops arctica (Lütken 1891)

Thirty-one specimens, 1.9–4.4 cm, between 175 and 185 fath, 1400–1800 hr, July 1967, by Isaacs-Kidd net south of La Have and Emerald banks. Bottom depth 300–350 fath.

This species is recorded once previously from the Canadian region, a specimen being taken by the Michael Sars expedition of 1910 (Bolin, 1959) northeast of Flemish Cap over bottom depths of 1000–2000 fath.

Hygophum hygomi (Lütken 1892)

Fifty-three specimens, 3.5–5.6 cm, between the surface and 55 fath, 2300-0200 hr, July 1968, by Isaacs-Kidd net south of La Have Bank. Bottom depth 660–715 fath. Previously unrecorded from the Canadian region.

Hygophum macrochir (Günther 1864)

One specimen, 2.7 cm, at 10 fath, 1800 hr, October 1968, by Isaacs-Kidd net south of Sable Island Bank. Bottom depth 400–510 fath. Previously unrecorded from the Canadian region.

Lampanyctus alatus Goode and Bean 1895

One specimen, 5.1 cm, at 220 fath, 1800 hr, February 1968, by Isaacs-Kidd net southeast of La Have Bank. Bottom depth 335 fath. Previously unrecorded from the Canadian region.

Lampanyctus festivus Tåning 1928

One specimen, 3.7 cm, at 50 fath, 2000 hr, October 1968, by Isaacs-Kidd net south of Sable Island Bank. Bottom depth 460–520 fath. Previously unrecorded from the Canadian region.

Lampanyctus pusillus (Johnson 1890)

Two specimens, 2.1 and 3.5 cm, at 50 fath, 1900 hr, October 1968, by Isaacs-Kidd net south of Sable Island Bank. Bottom depth 500–520 fath. Previously unrecorded from the Canadian region.

Lobianchia dofleini (Zugmayer 1911)

Four specimens, 2.3–3.2 cm, between 25 and 50 fath, 1800–0200 hr, July and October 1968, by Isaacs-Kidd net south of La Have and Sable Island banks. Bottom depths 480–695 fath.

Nafpaktitis (1968) records over 1500 specimens from the Slope Water region south of Nova Scotia, and Hersey and Backus (1954) found this species to be the most common myctophid in collections off southern New England.

Myctophum punctatum Rafinesque 1810, spotted lanternfish

One hundred and eight-two specimens, 2.3–9.4 cm. Ninety-two of these taken at the surface by neuston and 1-m nets and 51 in oblique Isaacs-Kidd tows, 0100–0730 hr in August 1968, northeast of the Magdalen Islands over depths of 155–250 fath. Thirty-three were taken between surface and 260 fath in July 1967, and February, July, and October 1968, by Isaacs-Kidd net from southwest of La Have Bank to southeast of Sable Island Bank over bottom depths of 145–740 fath. Six taken by otter trawl: one in 160–220 fath in July 1967 and three in 200 fath in February 1968, southeast of La Have Bank, one in 200 fath, southwest of Sable Island Bank, and one in 158 fath, southwest of La Have Bank in January and February 1969.

Notolychnus valdiviae (Brauer 1904)

Three specimens, 1.8–1.9 cm, between 55 and 110 fath, 2200–2400 hr, July 1968, by Isaacs-Kidd net south of La Have Bank. Bottom depth 380–740 fath. Previously unrecorded from the Canadian region.

Notoscopelus krøyeri (Malm 1863), Krøyer's lanternfish

Twenty-five specimens, 2.3–13.0 cm, between the surface and 240 fath in July 1967, July and October 1968, and January 1969, by Isaacs-Kidd net south of La Have and Sable Island banks. Bottom depths 255–715 fath. A further specimen, 10.3 cm, from the stomach of a silver hake caught southwest of La Have Bank in 195 fath, in February 1969.

Notoscopelus resplendens (Richardson 1844)

Two specimens, 4.5 and 4.9 cm, at 10 fath, 0100 hr, July 1968, by Isaacs-Kidd net south of La Have Bank. Bottom depth 700 fath. Previously unrecorded from the Canadian region.

Symbolophorus veranyi (Moreau 1888), largescale lanternfish

Eight specimens, 3.3–6.1 cm, between surface and 55 fath, 2300–0200 hr, July 1968, by Isaacs-Kidd net south of La Have Bank. Bottom depth 660–715 fath.

Family PARALEPIDIDAE

Notolepis rissoi krøyeri (Lütken 1891), white barracudina

Sixteen specimens, 1.9–10.4 cm, between 35 and 220 fath in May and July 1967, February and July 1968, and January 1969, by Isaacs-Kidd net from south of La Have Bank to southwest of Sable Island Bank. Bottom depth 150–740 fath.

ORDER Apodes

Family NEMICHTHYIDAE

Nemichthys scolopaceus Richardson 1848, Atlantic snipe eel

Thirty-two specimens, 28.2+ to 95.8+ cm, between surface and 115 fath, 1900–0200 hr, July and October 1968, by Isaacs-Kidd net south of La Have and Sable Island banks.

Family NESSORHAMPHIDAE

Nessorhamphus ingolfianus (Schmidt 1912)

One specimen, 19.5 cm, at 85 fath, 2300 hr, July 1968, by Isaacs-Kidd net south of La Have Bank. Bottom depth 480–645 fath.

Rees (1964) first recorded this species from the Canadian region, taking three specimens southwest of Sable Island Bank and one on the southwestern slope of Grand Bank.

ORDER Berycomorphi

Family POLYMIXIIDAE

Polymixia lowei Günther 1859, beardfish

One specimen, 6.7 cm, taken by otter trawl in 93–94 fath, southwest of Browns Bank (42°30′N, 66°01′W) in July 1968.

This record extends the species range 240 miles to the northeast, the previous northern limits being off Long Island, New York (Lachner, 1955).

ORDER Percomorphi

Family CARANGIDAE

Trachurus trachurus (Linnaeus 1758), horse mackerel

Two specimens, 5.8 and 6.0 cm, by otter trawl in 64–92 fath in July 1968; one from northeastern Georges Bank (41°30′N, 66°03′W), one from southeast of Browns Bank (42°18′N, 65°10′W).

This species has previously been recorded on three occasions from the Gulf of Maine (Bigelow and Schroeder, 1953).

Decapterus macarellus (Cuvier 1833,) mackerel scad

One specimen, 5.2 cm, by otter trawl in 64–69 fath, on northeastern Georges Bank (41°30′N, 66°03′W) in July 1968.

Family SPARIDAE

Stenotomus chrysops (Linnaeus 1766), scup

One specimen, 8.5 cm, taken in 48 fath by a commercial otter trawler on the Murr Ledges, south of Grand Manan, in October 1968.

Family ZOARCIDAE

Lycodes esmarki (Collett 1875), Esmark's eelpout

One specimen, 25.7 cm, in 158 fath southwest of La Have Bank by otter trawl in February 1969.

Family CENTROLOPHIDAE

Centrolophus niger (Gmelin 1788), black ruff

One specimen, 31.6 cm, captured by the commercial otter trawler *P. J. Lawrence* in 235 fath southeast of La Have Bank (42°46′N, 63°43′W) on August 24, 1968.

Templeman and Haedrich (1966) first recorded this species in Canadian waters from Sable Island Bank and the southeastern slope of Grand Bank.

Family NOMEIDAE

Psenes maculatus Lütken 1880, silver driftfish

One specimen, 4.3 cm, at 55 fath, 2400 hr, July 1968, by Isaacs-Kidd net south of La Have Bank. Bottom depth 660–675 fath. Not previously recorded from the Canadian region.

ORDER Scleroparei

Family SCORPAENIDAE

Helicolenus dactylopterus (De la Roche 1809), blackbelly rosefish

Two specimens, 2.8 and 3.2 cm. One taken at 160 fath by Isaacs-Kidd net southeast of La Have Bank in July 1967. Bottom depth 360 fath. One taken by otter trawl in 86–92 fath, southeast of Browns Bank in July 1968.

Family COTTIDAE

Icelus bicornis (Reinhardt 1841), twohorn sculpin

One specimen, 4.7 cm, taken by otter trawl in 86–92 fath, southeast of Browns Bank in July 1968. Bottom temperature 8.5 C.

The southern limit in Canadian waters of this arctic species was previously considered to be Ungava Bay (Leim and Scott, 1966). The present find gives credence to disputed records from the Gulf of St. Lawrence (Dannevig, 1919).

DEPTH DISTRIBUTION

Extensive diurnal vertical migrations are undertaken by many mesopelagic species. In the present study, day tows were made to depths greater than 250 fath and night tows only to 125 fath, yet night tows contained more individuals and showed greater species diversity, strongly suggesting that at least part of the populations of many of these species perform diurnal vertical migrations. Such migrations were made by six out of seven species that occurred in both day and night tows off the Nova Scotia banks (Table 1). *Chauliodus sloani*, *M. punctatum*, and *N. krøyeri* did not occur above 200 fath during the day, yet were not uncommon above 100 fath at night. *Ceratoscopelus maderensis* did not occur above 150 fath, nor *M. muelleri* and *S. boa ferox* above 100 fath in daylight, although all three were common between the surface and 150 fath at night.

Although it is not obvious from Table 1, *M. punctatum* is abundant at the very surface at night as is shown by the capture of 92 in surface nets in the Gulf of St. Lawrence. Of the species taken only at night, *H. hygomi* and *S. veranyi* were also most common at the surface.

DISCUSSION

Thirty-five species are recorded, 25 of which are mesopelagic fishes, indicating the lack of attention given this part of the fish fauna. Of the 10 species new to Canadian waters, eight are mesopelagic.

Bathythermograph traces indicate that the Isaacs-Kidd tows, which captured these mesopelagic fishes, were made in water with the temperature characteristics of Coastal Water, as the minimum temperatures above 140 fath were in all instances well below 10 C (McLellan et al., 1953). However, inshore water bodies cannot be expected to have distinctive mesopelagic faunas, and the fauna sampled here is probably characteristic of the Slope Water region that lies between the Coastal Water and the Gulf Stream. This Slope Water is formed of waters from the warm Gulf Stream and the cold Labrador Current (McLellan, 1957).

It is noteworthy that both arctic and tropical species are represented in this regional fauna and that it is dominated numerically by a subarctic-boreal species, *B. glaciale*. The abundance of northern species, such as *N. krøyeri*, *M. punctatum*, and *H. arctica*, as well as *B. glaciale*, would seem to indicate

TABLE 1. Diurnal distribution of some mesopelagic species taken by Isaacs-Kidd net off the Nova Scotia Banks. (D = day, N = night.)

Depth range of tows (*fath*):		Surface	5–50	50–100	100–150	150–200	200–250	250–300
Minutes fished:	D	–	90	150	420	510	330	30
	N	15	150	120	60	–	–	–
Notolepis rissoi krøyeri	D	–	1	3	1	2	4	–
	N	–	–	–	2	–	–	–
Maurolicus muelleri	D	–	–	–	1	–	1	–
	N	1	4	6	2	–	–	–
Stomias boa ferox	D	–	–	–	8	5	3	1
	N	4	17	24	5	–	–	–
Chauliodus sloani	D	–	–	–	–	–	2	2
	N	–	1	2	–	–	–	–
Ceratoscopelus maderensis	D	–	–	–	–	1	6	–
	N	–	30	25	4	–	–	–
Myctophum punctatum	D	–	–	–	–	–	2	1
	N	7	6	10	4	–	–	–
Notoscopelus krøyeri	D	–	–	–	–	–	1	–
	N	1	19	2	1	–	–	–

the strong influence of the Labrador Current in the region of the Central Scotian Shelf.

Tropical and subtropical species, such as *N. resplendens*, *H. macrochir*, and *D. termophilus*, swept north in the Gulf Stream, may be carried into the Slope Water in eddies that break away from the main Gulf Stream system (Hachey, 1961). The importance of the Gulf Stream in transporting southern species to colder northern waters is demonstrated by Nafpaktitis (1968) in his review of the myctophid genera *Lobianchia* and *Diaphus*. He records 11 species from the eastern Slope Water region (defined here as lying north of 41°N and west of 52°W)at least six of which were carried in the Gulf Stream from tropical waters. Of these 11 species only one, *Diaphus metopoclampus* (Cocco), had previously been recorded from the area (Vladykov, 1935 — as *Aethoprora effulgens* Goode and Bean — see Leim and Scott, 1966).

In the southwestern part of the Slope Water region, which extends to Cape Hatteras, northern forms such as *B. glaciale* apparently do not occur in abundance and the fauna is dominated by more southern species, e.g. *L. dofleini* (Hersey and Backus, 1954) and *C. maderensis* (Backus et al., 1968). However, both these species are also quite common in the eastern part of the Slope Water region. These faunal differences between eastern and western regions of the Slope Water parallel differences in physical and chemical characteristics, the water of the western region being more like Atlantic water than is that of the east (McLellan, 1957).

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Preliminary Survey of the Sublittoral Flora of Halifax County[1,2]

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Edelstein, T., J. S. Craigie, and J. McLachlan. 1969. Preliminary survey of the sublittoral flora of Halifax County. J. Fish. Res. Bd. Canada 26: 2703–2713.

During the autumn of 1965 and the summer of 1966, observations and sublittoral collections to a depth of 40 m were made of the benthic vegetation of the Atlantic coast of Halifax County. A total of 166 species (exclusive of crustose corallines) were recorded and distributed as 72 Rhodophyceae, 66 Phaeophyceae, 26 Chlorophyceae, 1 Xanthophyceae, and 1 Chrysophyceae. Thirty-three of these species have been recorded as new to Nova Scotia. Very few of the macrophytic species collected are exclusively sublittoral. Three distinct vegetational associations, each characteristic of a particular depth range, were recognized. From the sublittoral fringe to a depth of about 15 m, a *Laminaria–Desmarestia* association occurs. Below 10 m the flora is dominated by an *Agarum–Ptilota* association, and at 30–40 m a *Phyllophora–Polysiphonia* association is present. In some species morphological variations were correlated with depth.

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INTRODUCTION

Sublittoral observations based on dredging and drift material are incomplete, and accurate descriptions of vegetational associations and habitats are impossible. Since the early work of Gislén (1930), numerous underwater surveys and observations have been made. The use of scuba has accelerated these investigations, and, as examples, the works of Burrows (1958), Forster (1954, 1955), Kain (1961), McLean (1962), and Neushul (1965, 1967) may be cited.

The only comprehensive sublittoral survey in the northwestern Atlantic is that of Lamb and Zimmermann (1964) at Cape Ann, Massachusetts. Adey (1966) reported on the sublittoral coralline algae in Nova Scotian waters, and MacFarlane (1966) made a sublittoral survey, primarily for commercial species, in the Northumberland Strait. Colinvaux (1966) compared the vertical distribution of some of the more common species of St. Andrews, N.B., with those given by Lamb and Zimmermann (1964) and Wilce (1959), but she gave no clear indication of the source of her sublittoral information for the Bay of Fundy. Lund (1959) in eastern Greenland and Wilce (1959) in northwest Newfoundland have provided some information, obtained by dredging and drift material, of the sublittoral vegetation of these areas.

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[1] The seventh paper in a series on investigations of the marine algae of Nova Scotia.
[2] Issued as NRCC 10973.

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Recent work from this laboratory (Edelstein and McLachlan, 1967a, b; Edelstein et al., 1967) has been primarily surveys of the intertidal flora of Nova Scotia. In the present investigation we have extended these observations to the sublittoral of the Atlantic coast of Halifax County. A number of the species recorded in this preliminary survey have been reported as new to Nova Scotia (Edelstein and McLachlan, 1968, 1969).

AREAS SURVEYED

Two areas along the Atlantic coast of Nova Scotia, near the City of Halifax, were studied. Lawrencetown is east of Halifax Harbour and this area is overlaid by glacial till. There is a considerable outflow of fresh water from Porter's and Lawrencetown lakes. Our transect commenced at Lawrencetown Beach, where there are the remains of a drumlin severely cliffed by wave action, and proceeded SSW for a distance of 8 km to a depth of approximately 40 m (Fig. 1). The bottom slopes gently southward, and moderately strong, surging currents occur along this exposed coastline. The inshore sublittoral region consists of a sandy bottom that gives way rapidly to small to moderate sized stones of

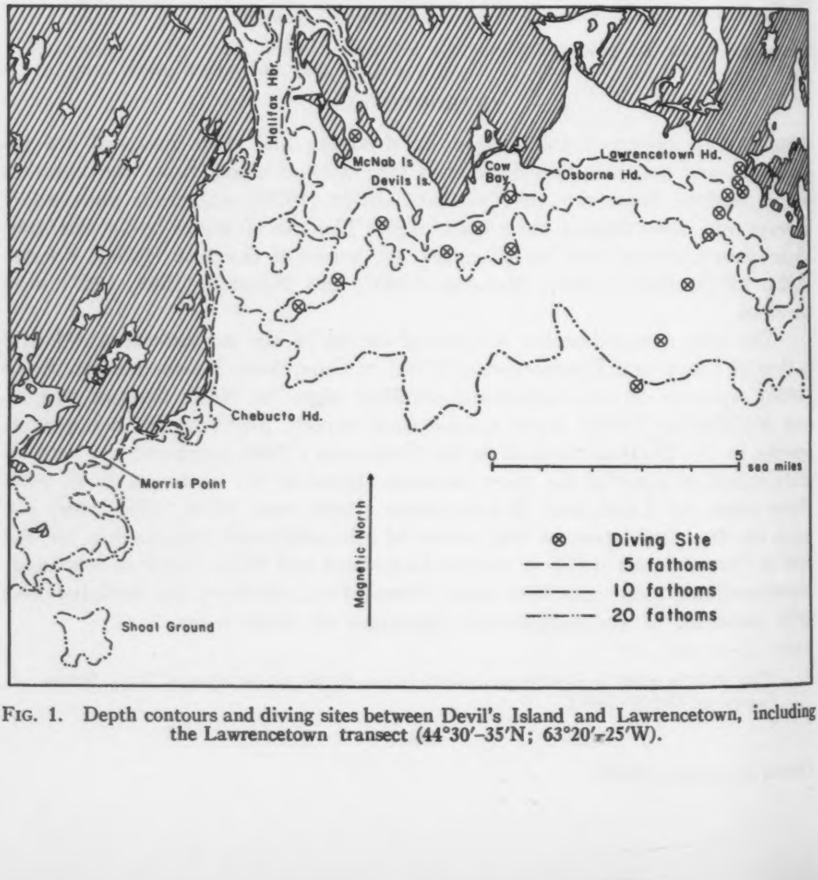

Halifax Hbr.
McNab Is
Devils Is.
Cow Bay
Lawrencetown Hd.
Osborne Hd.
Chebucto Hd.
Morris Point
Shoal Ground
Magnetic North
0
5
sea miles
⊗ Diving Site
5 fathoms
10 fathoms
20 fathoms

FIG. 1. Depth contours and diving sites between Devil's Island and Lawrencetown, including the Lawrencetown transect (44°30′–35′N; 63°20′–25′W).

quartzite, shale, and slate. Usually these rocks are partially embedded in sand, silt, and finely broken shells. Storm action results in a shifting of this unstable bottom from year to year, and the quartzite bedrock was observed only at the deeper stations.

A second transect, established about 24 km west of Lawrencetown Beach, commenced at Fink Cove and proceeded southward to a depth of 36 m on Shoal Ground (Fig. 2). In this area the substrate consists of boulders with occasional patches of gravel and stones, which overlie the granitic bedrock. Silt, sand, and freshwater runoff are negligible. The bottom profile is extremely irregular and subject to strong wave action.

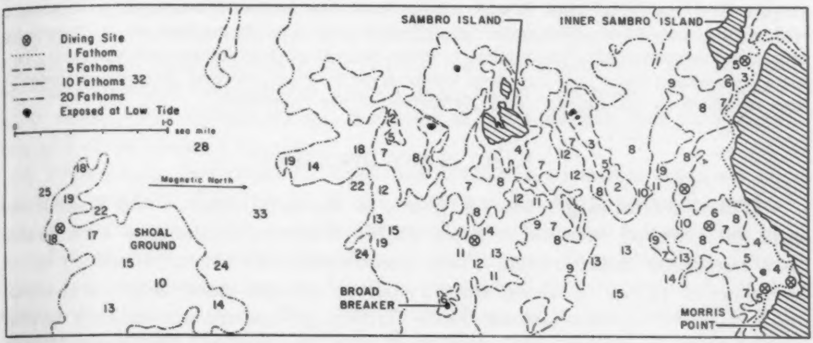

⊗ Diving Site
1 Fathom
5 Fathoms
10 Fathoms
20 Fathoms
● Exposed at Low Tide
0
1·0
sea mile
Magnetic North
SAMBRO ISLAND
INNER SAMBRO ISLAND
SHOAL GROUND
BROAD BREAKER
MORRIS POINT

FIG. 2. Depth contours and diving sites in the Fink Cove (Morris Point) transect (44°25′–30′N; 63°30′–35′W).

Between the Lawrencetown and Fink Cove transects, several additional sites were examined (Fig. 1).

In the area under consideration, regular hydrographic observations of the inshore waters are practically nonexistent. The available information on temperature and salinity, taken at a station approximately 1.1 km south of Chebucto Head, is summarized in Fig. 3.

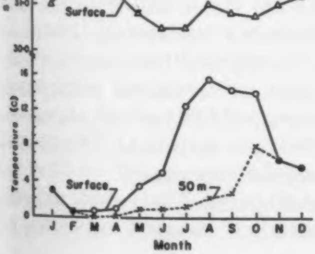


FIG. 3. Salinity and temperature data for water at the surface and at 50 m, taken at a station approximately 1.1 km south of Chebucto Head, Halifax County (abstracted from Taylor, MS, 1966).

MATERIALS AND METHODS

During the autumn of 1965 and the summer of 1966, a total of 41 dives, 6 of which exceeded a depth of 25 m, were made by divers using commercially available scuba equipment. At the shallower stations the divers spent 20–25 min on the bottom but at depths exceeding 25 m, the dives were limited to 10 min. Dives to depths that would have required decompression were not undertaken. Depths were determined either by a Ferrograph (Ferrograph Co. Ltd., London) recording depth-sounder or by a lead sounding line.

Large plants were detached from the substratum with a knife and placed in collecting sacks made from nylon meshing or plastic window screening. Delicate specimens were collected in polyethylene "snap-cap" vials. A large canvas basket was frequently employed to collect rocks with their attached flora. The basket was raised by means of the tender's winch. Immediately after the divers returned to the tender, a written account of their observations was made. Specimens were sorted and stored in a deep freezer prior to being processed. Voucher specimens are now deposited in the Marine Algal Herbarium of this laboratory.

RESULTS AND DISCUSSION

The common larger benthic plants in the sublittoral of the Halifax area may be grouped into three recognizable floristic associations each related to a particular depth range. These associations are characterized by an assemblage of plants in which certain species are dominant or more abundant than in the other associations. These floristic groups are immediately obvious to the observer, and may be compared with the striking littoral zonation in our area (Stephenson and Stephenson, 1954). As in the littoral area there is considerable overlap of species from one group to another. A tripartite sublittoral vegetative zonation has been noted in other areas (cf. Lamb and Zimmermann, 1964; Neushul, 1967) and may well be a general feature of cold waters.

A *Laminaria–Desmarestia* association occurs from the sublittoral fringe (*Alaria esculenta* band) to a depth of about 15 m. This association includes a large number of species and as many as 50 have been recorded in a single collection. In the uppermost range of this association, many plants such as species of *Monostroma*, *Ulva*, *Chordaria*, and *Porphyra* are littoral annuals as was noted previously by Lamb and Zimmermann (1964). These species occurred scattered, usually between patches of *Alaria* and *Laminaria*, and they tended to decrease in number and disappear where the *Laminaria* cover became dense. Similar observations have been made in other areas where a reduction in the number of species, attributed to shading, has been observed in dense kelp beds, and the understory vegetation is more characteristic of deeper waters (McLean, 1962; Neushul, 1965). On stable substrate the most common species of this association are *Laminaria saccharina*, *Laminaria longicruris*, *Laminaria digitata*, *Laminaria sp.* (a digitate form not yet identified), *Desmarestia viridis*, *Desmarestia aculeata*, *A. esculenta*, *Rhodymenia palmata*, *Saccorhiza dermatodea*, *Chondrus crispus*, *Cystoclonium purpureum*, *Ceramium rubrum*, *Polysiphonia urceolata*, *Corallina officinalis*, *Phyllophora brodiaei*, *Phyllophora membranifolia*, and encrusted corallines.

On less stable substrate such as small boulders, stones, and shells *Ahnfeltia plicata*, *Halopteris scoparia*, *Sphacelaria plumosa*, *P. brodiaei*, and *Polyides* *rotundus* were locally common. Extensive development of *Laminaria* did not occur on this type of substrate and Kain's (1962) observations are in general agreement with ours. As noted by others, the lower limit of *Laminaria* was approximately 15 m (cf. Lamb and Zimmermann, 1964; Kain, 1962; Forster, 1954, 1955).

Below 10 m an *Agarum–Ptilota* association was dominant and the more common species of this assemblage are *Agarum cribrosum*, *Ptilota serrata*, *Phycodrys rubens*, *Membranoptera alata*, *Peysonelia rosenvingii*, *Rhodophyllis dichotoma*, *Odonthalia dentata*, *Euthora cristata*, *Alaria grandifolia*, *Turnerella pennyi*, and encrusted corallines. The number of species present was reduced considerably, and most species of the littoral zone were absent. A few species from the *Laminaria–Desmarestia* group, such as *R. palmata*, *Phyllophora* spp., *D. viridis*, and *Laminaria* sp., occurred at these depths, but they were scarce and often reduced in size.

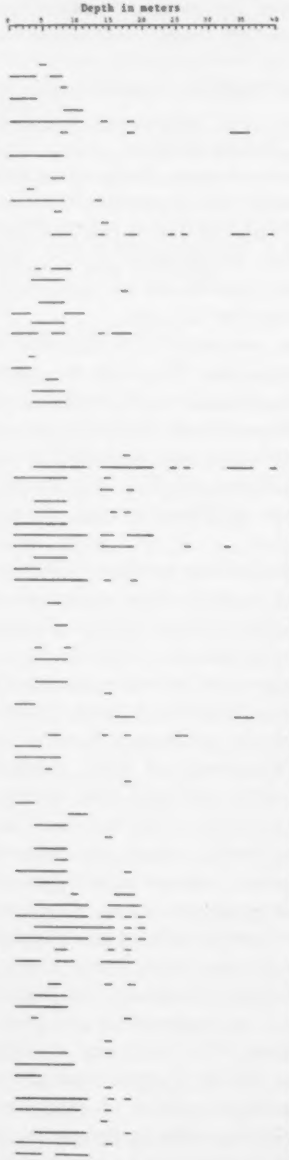
A *Phyllophora interrupta–Polysiphonia arctica* association occurred at the deepest stations (from 30 to 40 m) we observed. *Phyllophora interrupta* was found on the upper surfaces of boulders, whereas *P. arctica* formed mats on flat, rocky substrate covered with silt and was absent from the Fink Cove transect. Less conspicuous species found in this association were the delicate, deepwater form of *P. serrata* (Fig. 11), dwarf plants of *A. cribrosum* (Fig. 9), occasional specimens of *Dilsea integra*, *P. rubens*, and patches of encrusting corallines.

A detailed listing of the species collected together with reference to their vertical distribution, reproductive structures, and the host of endo- and epiphytes is given in Table 1. Distribution with depth of the more common species is illustrated diagrammatically (Fig. 4, 5).

Many of the larger species we encountered in this investigation are restricted to the sublittoral along the Atlantic coast of the province. However, elsewhere, and especially in the Bay of Fundy, most of these plants occur also in the littoral zone (Edelstein, *in herb.*; Edelstein et al., unpublished data). It seems therefore rather doubtful that many of our species are exclusively sublittoral. Neushul (1965) reported that a large percentage of the taxa he collected on the Pacific coast were known only from the sublittoral. He predicted that many more exclusively sublittoral species would be found when this area is investigated more completely.

In our area the lower limit for the majority of chlorophycean species was about 10 m, and only a few were found at greater depths down to 20 m. Two endophytes, *Chlorochytrium inclusum* and *Endocladia viridis*, were not influenced by depth other than that imposed by the host plant. Species of Phaeophyceae occurred down to about 20 m with only *A. cribrosum* and *D. viridis* penetrating to the deeper depths. Although most rhodophycean species were also encountered down to 20 m, a large number of plants were found in the deeper regions down to 40 m. We were unable to determine the lower limit of the algal vegetation, but Adey (1966) found that along the Nova Scotian coast certain species of crustose corallines were present at 70 m; the relative abundance of most

TABLE 1. Species collected in the sublittoral



Species		Host
Chlorophyceae		
Acrochaete repens		in *Chordaria*
Acrosiphonia arcta		
Chaetomorpha cannabina		
Chaetomorpha capillaris		
Chaetomorpha linum		
Chaetomorpha melagonium		
Chlorochytrium inclusum		in *Polyides* and *Membranoptera*
Chlorochytrium schmitzii		in *Ralfsia* and *Rhodophysema*
Cladophora crystallina		
Cladophora rupestris		
Cladophora sp.		
Ectochaete leptochaete		in *Ectocarpus*
Entocladia flustrae		in *Bryozoa*
Entocladia viridis		in *Membranoptera*, *Phycodrys* and *Phyllophora*
Monostroma fuscum		
Monostroma grevillei		
Monostroma leptodermum		
Monostroma oxyspermum		
Monostroma pulchrum		
Protoderma marina		
Pseudopringsheimia confluens		on *Saccorhiza* and *Laminaria*
Rhizoclonium riparium		
Spongomorpha aeruginosa		
Spongomorpha spinescens		
Spongomorpha sp.		
Ulva lactuca		
Phaeophyceae		
Acrothrix novae-angliae	Us	
Agarum cribrosum		
Alaria esculenta	Us	
Alaria grandifolia	Us	
Chorda filum		
Chorda tomentosum	Us	
Chordaria flagelliformis	Us	
Desmarestia aculeata	Us	
Desmarestia viridis		
Desmotrichum undulatum	Ps	
Dictyosiphon foeniculaceus		
Ectocarpus confervoides	Ps; Us	on *Laminaria*
Ectocarpus confervoides var. *arcta*	Ps; Us	on *Saccorhiza*
Ectocarpus confervoides var. *crouanii* ?	Ps; Us	
Ectocarpus confervoides var. *siliculosus*	Ps; Us	
Ectocarpus fasciculatus	Us	
Ectocarpus fasciculatus var. *fasciculatus*	Ps; Us	
Ectocarpus sp.		
Elachista fucicola		
Endodictyon infestans	Ps	in sponge and Bryozoa
Entonema aecidioides	Ps; Us	in *Laminaria*
Entonema aequale ?		in *Polyides*
Entonema alariae	Ps	on *Alaria*
Entonema effusum		in *Ceramium*
Feldmannia irregularis	Ps	
Fucus distichus subsp. *edentatus*	R	
Fucus vesiculosus		
Giffordia mitchellae ?	Ps	
Giffordia ovata	Ps	
Giffordia recurvata	Ps; Us	on *Saccorhiza*
Giffordia sp.	Ps; Us	
Haplospora globosa	M	
Hecatonema maculans	Ps	on *Rhodymenia*
Herponema desmarestiae	Ps	on *Desmarestia*
Isthmoplea sphaerophora	Ps; Us	on *Rhodymenia*
Laminaria digitata		
Laminaria longicruris		
Laminaria saccharina		
Laminaria sp. (digitate form)		
Laminariocolax tomentosoides	Ps	on *Laminaria* and *Saccorhiza*
Leptonematella fasiculata	Ps	on *Laminaria*
Lithoderma extensum ?		
Mikrosyphar polysiphoniae	Us	in *Polysiphonia*
Myrionema balticum	Ps	on *Rhodomela*, *Saccorhiza*
Myrionema corunnae		on *Laminaria*
Myrionema sp.	Ps	on *Odonthalia*
Petalonia fascia	Ps	
Phaeostroma pustulosum ?	Ps	on *Alaria*
Protectocarpus speciosus ?	Ps	on *Corallina*
Pilayella littoralis	Ps; Us	on *Laminaria*
Pilayella varia	Ps; Us	on *Saccorhiza*
Ralfsia clavata	Us	
Ralfsia verrucosa	Us	
Saccorhiza dermatodea	Us	
Scytosiphon lomentarius	Ps	
Sorapion kjellmanii		on *Rhodymenia*, *Zostera* and shells
Sorocarpus micromorus	Ps	
Sphacelaria cirrosa		

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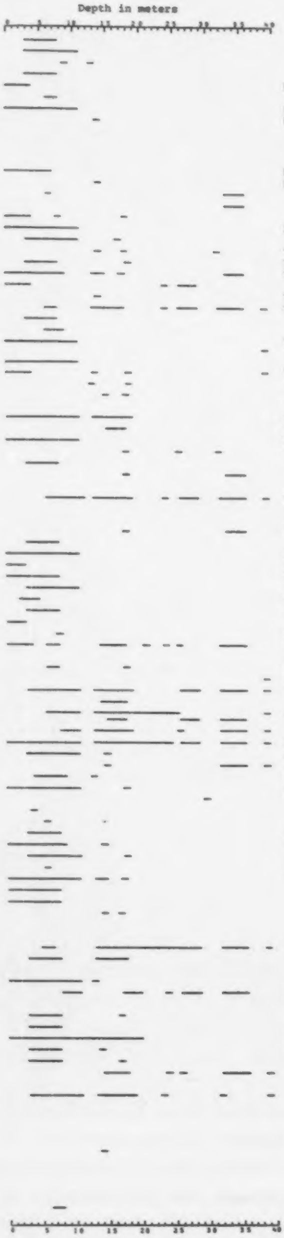
[a] M = monospores; T = tetraspores; Cy = cystocarps; Us = unilocular sporangia;
B = bispores.

[b] A filamentous phase in the life cycle of *Porphyra* and *Bangia*.

[c] The tetrasporic phase of *Bonnemaisonia hamifera*.

al distribution, reproductive structure, and host.[a]



Species		Host
Sphacelaria plumigera		
Sphacelaria plumosa		
Sphacelaria plumula		
Sphaerotrichia divaricata		on *Zostera*
Spongonema tomentosum	Ps	
Streblonema chordariae		in *Chordaria*
Streblonema parasiticum	Ps	in *Cystoclonium*
Streblonema sp.	Us	in *Odonthalia*
Rhodophyceae		
Acrochaetium alariae	M	on *Alaria*
Acrochaetium alcyonidii	M	on *Bryozoa*
Acrochaetium endozoicum		in a sponge
Acrochaetium hylinii ?		in *Phycodrys*
Acrochaetium polyides		in *Polyides*
Ahnfeltia plicata	N	
Antithamnion americanum		
Antithamnion boreale	T	
Antithamnion cruciatum	T	
Antithamnion floccosum	T	
Antithamnion pylaisaei		
Audouinella efflorescens	T; Cy	on *Odonthalia*
Audouinella membranacea	T	in *Bryozoa*
Bangia ciliaris		on *Rhodomela*
Bangia fuscopurpurea		
Ceramium rubrum	T	
Ceratocolax hartzii		on *Phyllophora*
Chondrus crispus		
Choreocolax odonthaliae	T; S	on *Odonthalia*
Choreocolax rabenhorstii ?		on *Phycodrys*
Conchocelis rosea[b]		in *Bryozoa* and *Spirorbis*
Corallina officinalis	B	
Cruoriopsis ensis ?		on *Desmarestia*
Cystoclonium purpureum	T	
Dilsea integra		
Dumontia incrassata		
Erythrocladia irregularis		on *Phycodrys* and *Membranoptera*
Euthora cristata	T; Cy	on *Ptilota* and Ascidian
Euthora cristata v. *angustata*	T; Cy	
Gonimotrichum alsidii		on *Antithamnion*
Halosaccion ramentaceum	T	
Halosacciocolax kjellmanii	T	on *Halosaccion*
Harveyella mirabilis	T; Cy	on *Rhodomela*
Hildenbrandia prototypus		
Kylinia collopoda	M	on *Chordaria*
Kylinia compacta		on *Polysiphonia*
Kylinia cytophaga		on *Porphyra*
Kylinia virgatula	M	on *Rhodymenia*
Melobesia farinosa	Co	on *Ptilota*, and *Phyllophora*
Melobesia lejolisii	Cy	on *Ptilota*
Melobesia zonalis	Cy	on *Phyllophora*
Membranoptera alata	T; Cy	on *Ptilota*
Membranoptera denticulata		on *Ptilota*
Odonthalia dentata	T; Cy	
Pantoneura baerii		on *Ptilota*
Peyssonnelia rosenvingii		
Phycodrys rubens	T;Cy	
Phyllophora brodiaei	N	
Phyllophora interrupta	N	
Phyllophora membranifolia	Cy	
Polyides rotundus	N	
Polysiphonia arctica	T	
Polysiphonia elongata ?		
Polysiphonia fibrillosa		
Polysiphonia flexicaulis	Cy	
Polysiphonia harveyi	T	
Polysiphonia nigrescens	T;Cy;S	
Polysiphonia novae-angliae	T;Cy	on *Chorda*
Polysiphonia urceolata	T	
Porphyra miniata		
Porphyra umbilicalis		
Porphyropsis coccinea		on *Polysiphonia*, *Rhodymenia* and *Odonthalia*
Ptilota serrata	T;Cy	
Rhodochorton penicilliforme	T;M	on *Ptilota* and *Laminaria*
Rhodomela confervoides	T;Cy;S	
Rhodophyllis dichotoma	Cy	on *Ptilota* and Ascidian
Rhodophysema elegans	T	
Rhodophysema parasitica	T	on *Laminaria*
Rhodymenia palmata	T	
Rhodymeniocolax sp. ?	T	on *Rhodymenia*
Trailliella intricata[a]		on *Corallina*
Turnerella pennyi	Cy	
Encrusting corallines		
Xanthophyceae		
Vaucheria sp.		
Chrysophyceae		
Phaeosaccion collinsii		

cular sporangia; N = nemathecia; R = receptacles; Co = conceptacles; S = spermatia;

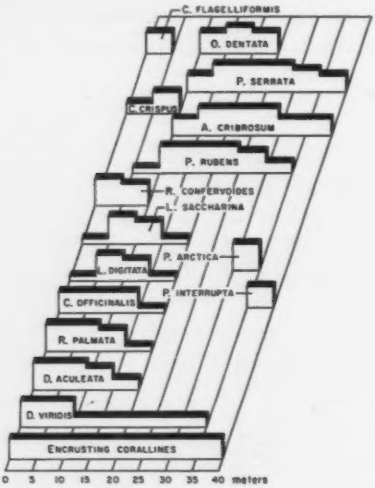

C. FLAGELLIFORMIS
O. DENTATA
P. SERRATA
C. CRISPUS
A. CRIBROSUM
P. RUBENS
R. CONFERVOIDES
L. SACCHARINA
P. ARCTICA
L. DIGITATA
P. INTERRUPTA
C. OFFICINALIS
R. PALMATA
D. ACULEATA
D. VIRIDIS
ENCRUSTING CORALLINES
0 5 10 15 20 25 30 35 40 meters

FIG. 4. Diagrammatic illustration of the vertical distribution of the main species at Lawrencetown with a visual approximation of their relative abundance.

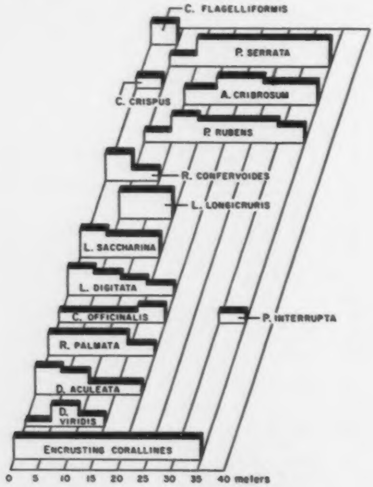
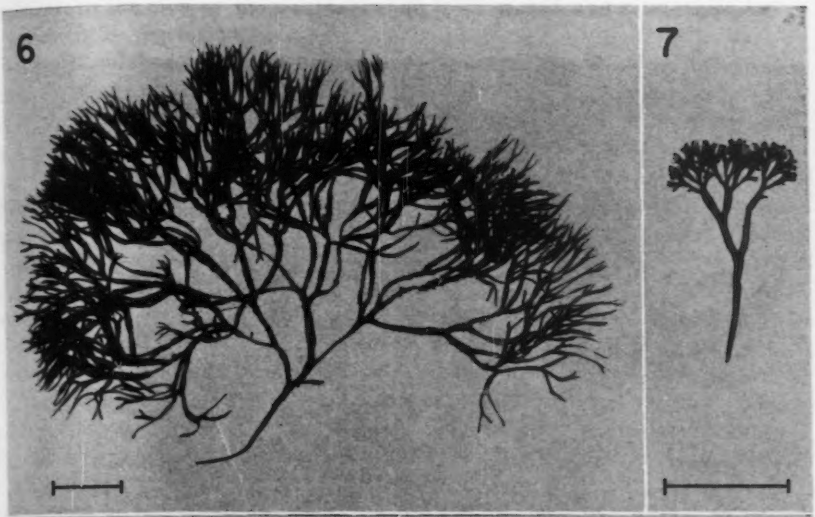
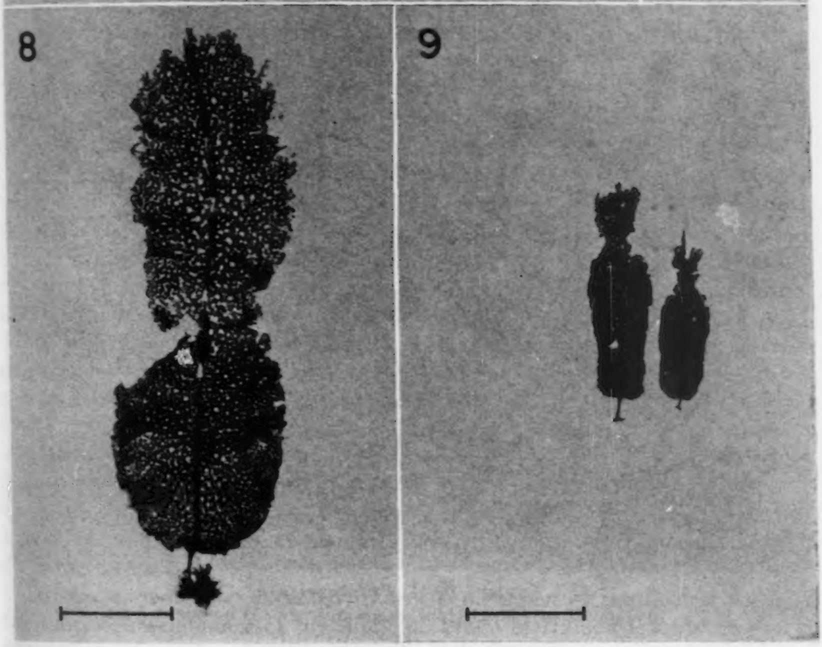

C. FLAGELLIFORMIS
P. SERRATA
C. CRISPUS
A. CRIBROSUM
P. RUBENS
R. CONFERVOIDES
L. LONGICRURIS
L. SACCHARINA
L. DIGITATA
C. OFFICINALIS
P. INTERRUPTA
R. PALMATA
D. ACULEATA
D. VIRIDIS
ENCRUSTING CORALLINES
0 5 10 15 20 25 30 35 40 meters

FIG. 5. The same as Fig. 4 for Fink Cove.

species decreased markedly at 30–40 m, however. Only rhodophycean species were found at the deeper depths, and similar observations have been reported from other areas (e.g., McLean 1962; Neushul 1965, 1967). Crustose coralline species are apparently able to inhabit appreciably deeper depths than are the fleshy forms.

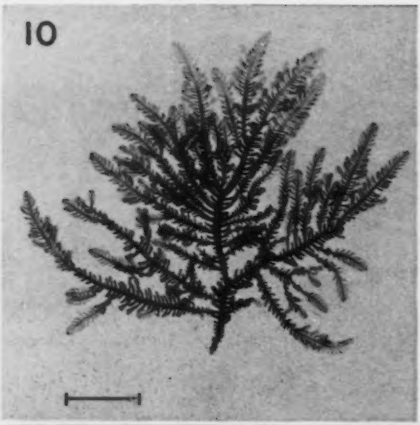
Large boulders and continuous rock were found heavily populated with many species, but relatively few mature plants occurred on small, movable stones or mussels. Species such as *P. arctica* and *O. dentata* were best developed in areas with some silt. *Ahnfeltia plicata* and *P. rotundus* occurred on stable

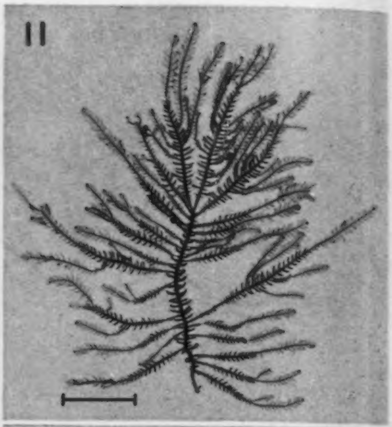


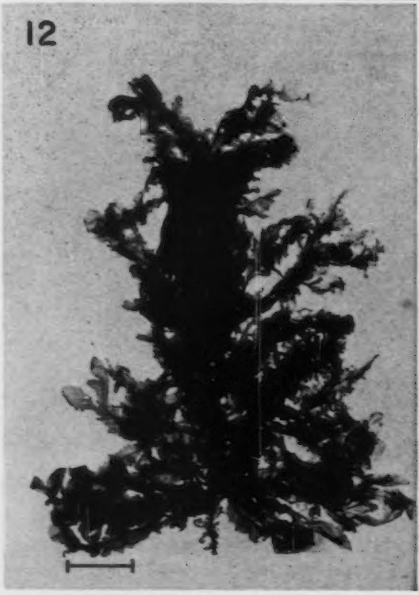


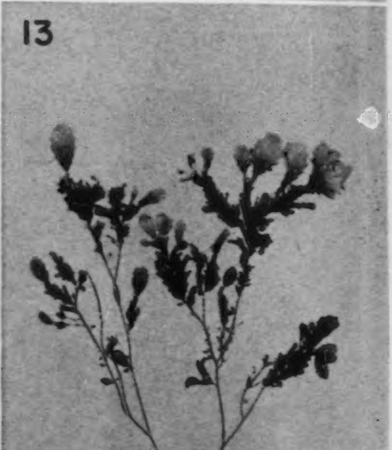
FIGURES 6–7. ***Chondrus crispus.***
Fig. 6: Deepwater form, September, 6 m; Fig. 7: Low-tidal form, January (bar = 5 cm).

FIGURES 8–9. ***Agarum cribrosum.***
Fig. 8: July, Fink Cove, 30 m; Fig. 9: June, Lawrencetown, 39 m (bar = 25 cm).









FIGURES 10–11. ***Ptilota serrata.***
Fig. 10: June, 20 m; Fig. 11: June, deepwater form, 31 m (bar = 5 cm).

FIGURES 12–13. ***Phycodrys rubens.***
Fig. 12: Epizoic on an ascidian, May, 25 m; Fig. 13: July, 35 m (bar = 5 cm).

substrate covered with sand or silt and flourished in the Lawrencetown environment. In the area we have investigated, mud, gravel, and sand supported no macroscopic vegetation.

A large number of microscopic species occurred as epi- or endophytes; others were epi- or endozoic, especially in sponges or bryozoans (Table 1) and at times densely covered the host. The only clear relationship between these species and their respective hosts appeared to be that of sharing the same depth range. The long stipes of the ascidian, *Halocynthia pyriformis*, which was common below 18 m, were found heavily clothed with *Phycodrys*, *Euthora*, *Ptilota*, *Rhodophyllis*, etc., all characteristic of that depth. Shells of deepwater molluscs were encrusted with a thick layer of corallines; such animals in shallower waters provided support for *Sphacelaria*, *Polyides*, and species common to the intertidal area. Scagel (1959) pointed out that the association between plants and animals in the sea is at times only a result of competition for space and a secure attachment.

Morphological variation of some species with depth was strikingly apparent. *Chondrus crispus* at 6–7 m was large with numerous fusiform branches (Fig. 6), whereas specimens from the intertidal were usually smaller, less branched with truncated tips (Fig. 7); similar observations have been noted by Newton et al. (1959). The deepwater form of *P. serrata* (Fig. 11) was delicate and very soft, and some specimens exceeded 30 cm in length. Width of the main branches, as well as the branchlets, was about one-half of that observed on plants of smaller but coarser forms in shallower water (Fig. 10). *Agarum cribrosum* was best developed at 15–30 m. In this depth range the plants were dark brown with fronds up to 300 μ thick, and specimens exceeding a meter in length were recorded (Fig. 8). Plants of this species from 39 m at Lawrencetown were small, up to 40 cm in length (Fig. 9), light-brown colored with a blade thickness of about 150 μ. Other species such as those of *Phycodrys*, *Euthora*, etc., also varied in size and form with depth. Unusually large specimens of *P. rubens* in the Fink Cove area were observed at 33–38 m (Fig. 13); much smaller plants were attached to the stipes of the ascidian (Fig. 12) at 25 m.

The flora of Fink Cove and Lawrencetown was found to be very similar. Less turbid conditions at Fink Cove permit the penetration of light to greater depths, correlating to a more abundant algal cover in deeper water. In addition, some species were observed at greater depths at Fink Cove than at Lawrencetown. Large plants of *Agarum* were taken from 32 m, and *Laminaria* sp., *Corallina*, and *Rhodymenia* were found at 17–18 m. Luxuriant beds of *L. longicruris* occurred at Fink Cove, but this species was absent at Lawrencetown. On the other hand *P. arctica*, *O. dentata*, *D. integra*, and *S. plumosa* were found only in the latter area.

Perhaps, as would be expected from a comparison of the littoral floras, there is a close resemblance of the sublittoral flora of Halifax County to that of Cape Ann, Massachusetts (cf. Lamb and Zimmermann, 1964). With few exceptions, our records include all of the species found at the Massachusetts site. The lower limit of our vegetation is at least twice that observed at Cape

Ann, but the shallowness of the vegetation there is probably due to an unstable substrate at the deeper depths (I. M. Lamb, personal communication). The occurrence in our sublittoral flora of many species considered subarctic or arctic indicates a close similarity to the northern flora, as has been described by Lund (1959) and Wilce (1959).

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Some Effects of Simulated Long-Term Environmental Fluctuations on Maximum Sustained Yield

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For a variety of stock recruit systems, in which environmental variability is simulated by random normal deviates used as multipliers or divisors, Ricker (J. Fish. Res. Bd. Canada 15: 991–1006, 1958) and Larkin and Ricker (J. Fish. Res. Bd. Canada 21: 1–7, 1964) demonstrated the benefits of complete stabilization of escapement as opposed to removal of a fixed proportion of the stock each year. The present paper is primarily concerned with the response of these same systems to a pattern of stochastic modification that is more regular in form, a pattern such as might be imagined to result from long-term trends in environmental conditions. The simulations indicate that the gains derived from complete stabilization of escapement are determined solely by the variance of the modifiers — thus the pattern of modification, i.e., long- or short-term, is relevant only in terms of its influence on the variance. In addition, a check for maximum equilibrium catch using catch statistics is described.

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BASIC SYSTEMS

THE CURVES USED in the present simulations are identical to those described by Ricker (1958). Curves A, B, and C are members of the exponential family $Z_t = we^{a(1-w)}$ where Z_t is production in year t, w is spawning stock size,[1] and a is a parameter determining maximum production and, consequently, the shape of the reproduction curve. For curves A, B, and C the parameter a assumes a value of 1.000, 2.000, and 2.678 respectively (Fig. 1).

Curve A describes the situation where maximum production occurs at the equilibrium position $w = 1.000 = Z_{\max}$. Displacements to the left of equilibrium ($w < 1.0$) result in a "climbing" of the ascending limb of the curve. Displacements to the right of equilibrium ($w > 1.0$) result in an immediate

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[1] All values are expressed in stock units, where one stock unit is the number of individuals associated with the unexploited equilibrium position.

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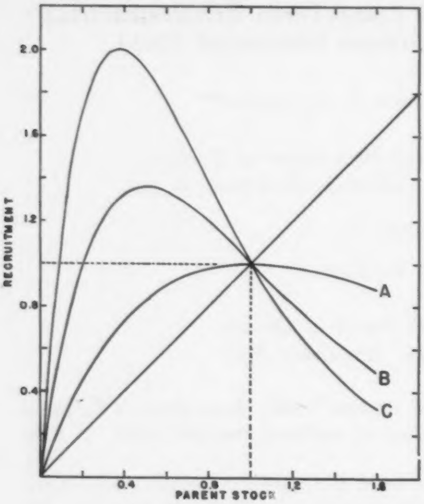

FIG. 1. Stock recruitment curves A, B, C of Ricker (1958) in which $z = we^{a(1-w)}$ for $a = 1.000$, 2.000, and 2.678, respectively.

compensation, the stock being depressed to a level below equilibrium density to which it gradually returns.

In curve B the maximum production is associated with a stock density of approximately one-third that of equilibrium density. Production per spawner at any given stock density is greater in curve B for $0 < w < 1$, and smaller for $w > 1$, than in curve A. Arbitrary displacement from equilibrium results in the production of a damped oscillation of abundance, which returns the stock to the equilibrium position.

Curve C is a more extreme version of curve B in that production rates are greater and compensation is more severe. The system is unstable in the sense that displacements from the equilibrium position result in the production of permanent oscillations and there is no tendency for the stock to return to the equilibrium of $w = 1$.

Curves F, G, and H (Fig. 2) each belong to a different family of curves. Curve H represents the converted Beverton–Holt relationship as described in Ricker (1958), and curves F and G are contrived equations representative of other possible types of stock recruit systems.

Curve F is characterized by an ascending limb that conforms to curve B for $0 < Z < 1$, coupled with an asymptotic part that maintains the stock at the equilibrium density for all other values of w.

Curve G may be considered as a representative of systems influenced by depensatory mortality factors, i.e., factors that are proportionately more severe at lower stock densities. The curve is identical to A except for $w < 0.4$, in which case production is calculated by $Z = we^{.433w}$.

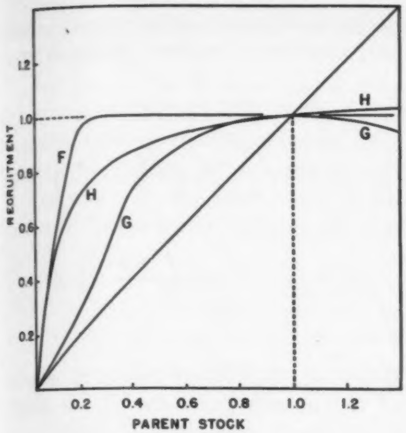

FIG. 2. Stock recruitment curves F, G, H of Ricker (1958). See text for explanation.

SIMULATION PROCEDURE

The procedure of simulation was as described by Larkin and Ricker (1964) with modifications for the long-term effects. Representing long-term fluctuations by a sin wave, for each generation, taken to be a year, the modifier is

$$Q = P \sin\left[\frac{m}{l} \cdot 2\pi\right]$$

where m is the year in a cycle and l is the cycle length. Thus, for the second year of a 6-year cycle the sin of $\frac{m}{l} \cdot 2\pi$ becomes the sin of $\frac{2}{6}(2\pi)$. This is scaled by the multiplier P to define amplitude, augmented by one, and used as a multiplier or divisor depending on sign.

Depending upon what is envisaged, there are various ways in which the sin function might be coupled with random normal deviates in the simulation of long-term fluctuations with superimposed random fluctuations. In addition to using either effect separately, two alternatives were provided for combined effects:

(1) Sin plus random — the value of Q was added to the value of a random normal deviate, after each was scaled to a desired order of magnitude. Then the absolute value was augmented by one and, depending on the sign, used as a multiplier or divisor. This representation might be appropriate for a situation in which both effects were visualized as occurring in the same environment.

(2) Sin times random — the value of Q was augmented by one, used as a multiplier or divisor, then was followed by a random normal deviate used in the same way. This type of simulation might be taken to represent the

situation in which the effects were occurring in different environments — say, for example, long-term effects in the sea; short-term random effects in fresh water.

Algebraically, the sin wave modified system may be written

$$Z_t = \left[w_t e^{a(1-w_t)} \left(1 + P \sin \left(\frac{m_t 2\pi}{l}\right)\right)\right]; \quad m = 1, \ldots \frac{l}{2}; \ \sin\left(\frac{m_t 2\pi}{l}\right) \geq 0$$

$$= \left[w_t e^{a(1-w_t)} \left(\frac{1}{1 + P\left|\sin \ \frac{m_t 2\pi}{l}\right|}\right)\right]; \quad m = \frac{l}{2}, \ldots l; \ \sin\left(\frac{m_t 2\pi}{l}\right) \leq 0$$

where Z_t = production in year t, w_t = spawning stock size in year t, m = year in sequence of cycle (i.e., m varies from 1 to 6 in each 6-year cycle), l = length of cycle in years, and P = scaling factor. For a fixed rate of fishing:

$$C_t = FZ_t$$

$$w_{t+1} = (1 - F)Z_t$$

where C_t = catch in year t, and F = fixed rate of fishing. For complete stabilization of escapement:

$$C_t = Z_t - w_m; \quad Z_t > w_m$$

$$C_t = 0; \quad Z_t < w_m$$

$$Z_{t+1} = w_m e^{a(1-w_m)}; \quad Z_t - w_m > 0$$

where w_m = escapement associated with maximum equilibrium catch (m.e.c.).

The computer simulations conducted were all run for 200 simulated years for all combinations of: (1) four long-term cycle lengths: 6, 11, 12, 24 years; (2) six stock recruit curves: A, B, C, F, G, H; (3) two levels of random effects; (4) two levels of long-term effects; (5) three patterns of fishing: fixed percentage, partial stabilization of escapement, and complete stabilization of escapement (Ricker, 1958); and (6) three patterns of environmental effects: sin effects only, sin plus random effects, sin times random effects.

DETERMINISTIC SIN EFFECTS

In the simulations using the sin wave modifier and a fixed percentage fishery, the catch pattern was initially one of erratic changes in abundance. Within a few generations a repeatable pattern of catch and escapement was evident, the values oscillating in phase with the sin modifier but at lower amplitudes. (The initial period of instability was a result of starting the simulations with the stock at its equilibrium density (w = 1)). Two levels of amplitude (P = .5, P = 2.0) were used in conjunction with four cycle lengths and the resulting mean catch and escapement values for 200 simulated years are summarized in Table 2, which can be compared with the simple deterministic case (Table 1).

For curves A, B, and C, the tabulated escapement values differ only slightly from the deterministic values associated with m.e.c.[2] The differences are attributable to the initial period of instability and the fixed 200 year period of simulation, which did not always end on a year completing a cycle.

For curve F, the mean catch is slightly less than the deterministic model and curve G goes to extinction under conditions of fixed percentage exploitation.

TABLE 1. Mean catch and escapement in deterministic models of six stock recruit relationships.

Curve	Catch	Escapement
A	0.330	0.433
B	0.935	0.361
C	1.656	0.314
F	0.760	0.210
G	0.330	0.433
H	0.520	0.240

TABLE 2. Mean catch and escapement in 200 year simulations of fixed percentage exploitation for each of six reproductive curve systems, with long-term environmental fluctuations, represented by sin modifiers, at cycle lengths of 6, 11, 12, and 24 years, and at two levels of amplitude. See text for explanation.

Cycle:		6 yr		11 yr		12 yr		24 yr	
Curve	Level	C[a]	E[b]	C	E	C	E	C	E
A	1	.333	.436	.333	.436	.336	.440	.340	.445
	2	.338	.442	.338	.442	.346	.453	.358	.469
B	1	.937	.360	.936	.360	.940	.362	.949	.365
	2	.948	.365	.945	.364	.956	.368	.981	.377
C	1	1.657	.313	1.656	.313	1.660	.314	1.676	.316
	2	1.673	.316	1.673	.316	1.684	.318	1.723	.325
F	1	.739	.204	.728	.201	.731	.202	.724	.200
	2	.746	.206	.711	.197	.742	.205	.750	.208
G[c]	1	.013	.018	.019	.024	.020	.026	.037	.049
	2	.014	.099	.026	.034	.028	.037	.057	.075
H	1	.543	.250	.550	.254	.553	.255	.562	.259
	2	.686	.317	.752	.347	.766	.354	.823	.380

[a] C = catch.

[b] E = escapement.

[c] Curve G goes to extinction in a few generations under conditions of fixed percentage exploitation.

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[2] The deterministic values of m.e.c. in Table 1 are determined by setting the first derivative of $w_t e^{(a-w_t)} - w_t$ equal to zero and solving for w.

Curve H is the only system that responds favorably to the combination of sin modification and fixed percentage exploitation. Curve H is similar to curve A in that maximum production is associated with a stock size much larger than the stock size that provides maximum catch, but differs in that there is no compensation at the higher stock densities. Since the catch is proportional to production in the fixed percentage fishery, gains are to be expected.

Table 3 summarizes the results of simulations using the same amplitude and cycle length modifications but with a stabilized fishery. Comparing these results with Table 1, it is apparent that a stabilized fishery results in an increased yield in all cases.

TABLE 3. Mean catch and escapement in 200 year simulations of a fishery with completely stabilized escapement for each of six reproductive curve systems, when long-term environmental fluctuations are represented by sin modifiers, at cycle lengths of 6, 11, 12, and 24 years and at two levels of amplitude. See text for explanation.

Cycle:		6 yr		11 yr		12 yr		24 yr	
Curve	Level	C[a]	E[b]	C	E	C	E	C	E
A	1	.368	.433	.367	.433	.369	.433	.375	.433
	2	.624	.370	.643	.351	.649	.348	.686	.330
B	1	.994	.361	.994	.361	.996	.361	1.007	.361
	2	1.422	.361	1.436	.361	1.442	.361	1.482	.361
C	1	1.744	.314	1.744	.314	1.748	.314	1.765	.314
	2	2.392	.314	2.414	.314	2.424	.314	2.487	.314
F	1	.837	.210	.837	.210	.839	.210	.847	.210
	2	1.169	.210	1.179	.210	1.184	.210	1.214	.210
G	1	.368	.433	.367	.433	.369	.433	.375	.433
	2	.394	.322	.312	.250	.311	.243	.236	.174
H	1	.557	.240	.556	.240	.558	.240	.564	.240
	2	.811	.240	.817	.240	.821	.240	.843	.240

[a] C = catch.
[b] E = escapement.

EFFECT OF CYCLE LENGTH AND AMPLITUDE MODIFICATIONS

In the previous section it was shown that the mean catches for curves A, B, and C are unchanged with the combination of sin modification and fixed percentage exploitation. Nevertheless, the pattern of the catch responds to both changes in cycle length and amplitude. Figure 3 compares, for curve C, the pattern of a 12-year cycle with that of a 24-year cycle; Fig. 4 shows the effect on a 12-year cycle of varying the amplitude of the sin modifier. For the fixed percentage exploitation, variation in cycle length has almost no effect on the standard deviation of catch (σ_c), and serves only to increase the length of the series of good and poor catches. As might be expected, σ_c responds to amplitude modifications, increasing as the amplitude becomes larger.

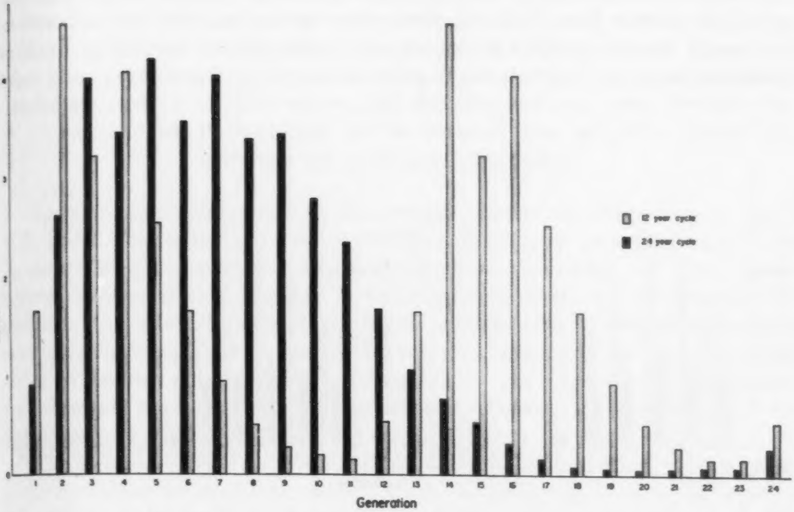

FIG. 3. Catch in deterministic simulation of a fixed percentage fishery on curve C systems with 12- and 24-year sin wave cycles for amplitude scaling factor P = 2.0.

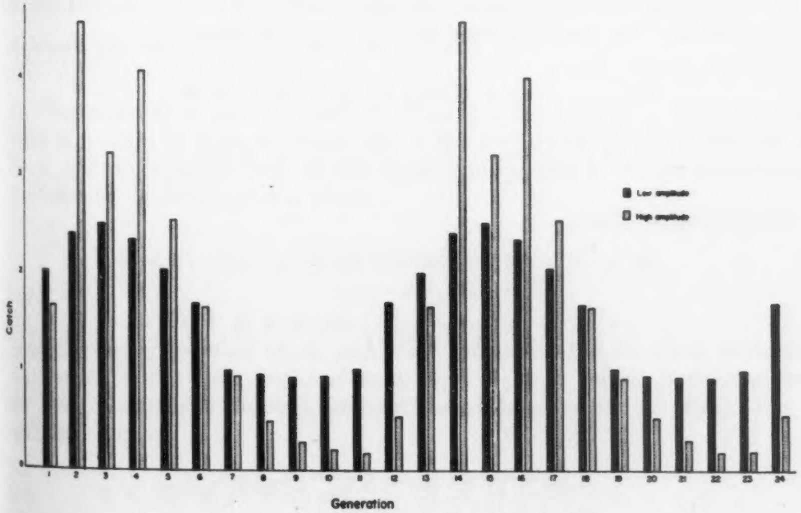

FIG. 4. Catch in deterministic simulation of a fixed percentage fishery on a curve C system with a 12-year sin cycle for amplitude scaling factor at two levels, P = 0.5 and P = 2.0.

For the stabilized fishery two slightly different catch patterns were evident. In the systems that had a relatively high production per spawner at low stock densities (curves B and C), the production never dropped below w_m and consequently the stocks always reproduced at an optimal rate (Fig. 5). In the less responsive systems (curves A and G) the catch pattern is typically a series of relatively good catches followed by a series of zero catches, the length of the series increasing as a function of the amplitude of the modifiers.

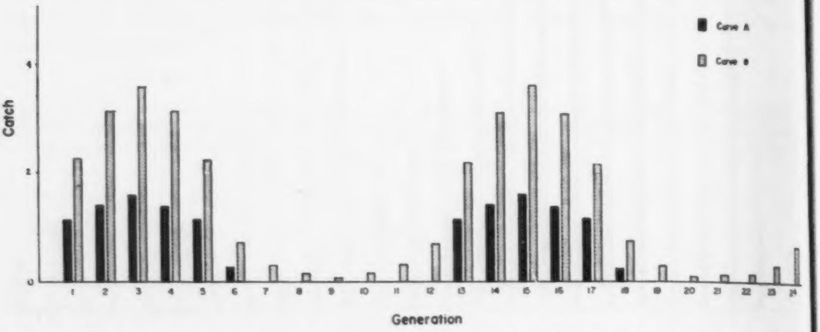

FIG. 5. Catch in deterministic simulation of a completely stabilized fishery on curve A and curve B systems with a 12-year sin cycle and amplitude scaling factor P = 2.0.

STOCHASTIC MODIFICATIONS

The deterministic sin modifications described above were stochasticized by either additive or multiplicative superimposition of random normal deviates. Algebraically, the modifications (R) may be represented as:

Additive effects

$$R = (1 + Q + \text{RND}) \text{ for } Q + \text{RND} > 0$$

$$= \left(\frac{1}{1 + |Q + \text{RND}|}\right) \text{ for } Q + \text{RND} < 0$$

Multiplicative effects

$$R = (1 + Q)(1 + \text{RND}) \text{ for } Q > 0, \text{ RND} > 0$$

$$= (1 + Q)\left(\frac{1}{1 + \text{RND}}\right) \text{ for } Q > 0, \text{ RND} < 0$$

$$= \left(\frac{1}{1 + Q}\right)(1 + \text{RND}) \text{ for } Q < 0, \text{ RND} > 0$$

$$= \left(\frac{1}{1 + Q}\right)\left(\frac{1}{1 + \text{RND}}\right) \text{ for } Q < 0, \text{ RND} < 0$$

where Q = scaled sin modifier, and RND = scaled random modifier.

In general, the addition of the stochastic modifiers serves only to accentuate the basic patterns described for the deterministic sin effects, the frequency of zero catches becoming greater for the stabilized fishery, and σ_c becoming larger for fixed percentage exploitation. The catches for the stabilized fishery are larger as a function of σ_c although no changes are observed for fixed percentage exploitation.

NATURE OF THE MODIFICATIONS

From the preceding results three general conclusions pertaining to curves A, B, and C are evident: (1) for a stabilized fishery, an increase in yield over the deterministic maximum is apparent for all systems subjected to stochastic modifications, and this increase is commensurate with the variance of the modifiers; (2) the addition of stochastic modifiers to a system undergoing fixed percentage exploitation has no effect on the mean catch, its value remaining the same as the deterministic maximum; (3) the pattern of modification (i.e., sinoidal, random, etc.) is unimportant relative to the variance of the modifications.

The increased yields for the stabilized fishery can, in a sense, be attributed to the technique of stochastic modification. Augmenting by one the absolute value of the random variate and subsequently using it as a multiplier or divisor, has the effect of multiplying production by an average modifier that is greater than 1. Algebraically, if X is a symmetrically distributed variable with mean 0, the frequency of a particular positive variate X_i is the same as the frequency of the corresponding negative value. The appropriate modifiers would be $1 + X_i$ for the former and $\dfrac{1}{1 + |X_i|}$ for the latter.

The mean of a pair of these modifiers is greater than 1; therefore, the mean of a series of these modifiers ($\overline{R}$) is also greater than 1. For example, if $X_i = +1$, the corresponding multiplier $= 2$, and for $X_i = -1$, the appropriate multiplier $= \frac{1}{2}$. Furthermore, since

$$\frac{(1 + X) + \left(\dfrac{1}{1 + |X|}\right)}{2}$$

is an increasing function of X, and since increasing the standard deviation (σ_x) results in the more frequent occurrence of large values, it follows that the mean modifier $\overline{R}$ will also increase as a function of σ_x. Thus, for a stabilized fishery

$$\bar{c} = [w_m e^{a(1-w_m)}]\,\overline{R} - w_m \text{ for } Z_t \geq w_m$$

where $\bar{c}$ = mean catch, and w_m = escapement associated with m.e.c.; $\bar{c}$, then, is a linear increasing function of $\overline{R}$.

The case of the fixed percentage fishery is somewhat less straightforward. For curves A, B, and C average production per spawner is higher than the deterministic case, for the same reason given in the preceding paragraph. However, the mean catch is virtually identical to the deterministic maximum.

The sufficient conditions necessary for this result are demonstrable algebraically. For a fixed percentage fishery

$$c_t = [w_t e^{a(1-w_t)}]\ [\mathrm{R}]\ [\mathrm{F}]$$

where c_t = catch in generation t, w_t = spawning stock in generation t, R = stochastic modifier, and F = exploitation *rate* appropriate for m.e.c. $(1 - e^{-a(1-w_m)})$. Substituting the expression for F we have

$$c_t = [w_t e^{a(1-w_t)} - w_t e^{a(w_m - w_t)}]\ [\mathrm{R}].$$

Harvesting at the equilibrium rate implies that $\overline{w}_t = w_m$. Substituting w_m for w_t in the expression for catch, the expected catch in a time period becomes

$$c_t = \mathrm{R}_t w_m e^{a(1-w_m)} - \mathrm{R}_t\, w_m.$$

Because $\mathrm{R}_t w_m$ is the escapement, the expected catch in period $(t + 1)$ is

$$c_{t+1} = \mathrm{R}_{t+1}\ \mathrm{R}_t\ w_m\ e^{a(1-\mathrm{R}_t w_m)} - \mathrm{R}_{t+1}\ w_m$$

and in $(t + 2)$ is

$$c_{t+2} = \mathrm{R}_{t+2}\ \mathrm{R}_{t+1}\ \mathrm{R}_t\ w_m\ e^{a(1-\mathrm{R}_{t+1}\mathrm{R}_t w_m)} - \mathrm{R}_{t+2}\ w_m.$$

The successive product of R_{t+i} $(i = 1, n)$ is 1, and ignoring the value of the last modifier $\mathrm{R}_{t=n}$ it is apparent that

$$\bar{c} = w_m\ e^{a(1-w_m)} - w_m$$

which is the deterministic maximum.

Biologically, the mathematics imply that the production from any given number of spawners exposed to a varying environment will, on average, be larger than if conditions were constant at a mean value. In the stabilized fishery this gain in production is reflected by increased yields, whereas in the fixed percentage fishery any gain is eliminated by the subsequent compensatory response.

Because the standard deviation of catch (σ_c) and mean catch ($\bar{c}$) are both increasing functions of $\overline{\mathrm{R}}$, $\bar{c}$ and σ_c are correlated. This relationship provides a means of determining, in the absence of information concerning the exact shape of a reproduction curve, whether a particular escapement is appropriate for m.e.c. By expressing $\bar{c}$ in stock units for a series of escapements of approximately the same size, it may be assumed that variations in the resultant catch may be attributed to environmental factors acting independently of the density of the stock. Since σ_c and $\bar{c}$ are unique for a given optimal escapement and curve type (Fig. 6), it is possible to obtain a deterministic value of a associated

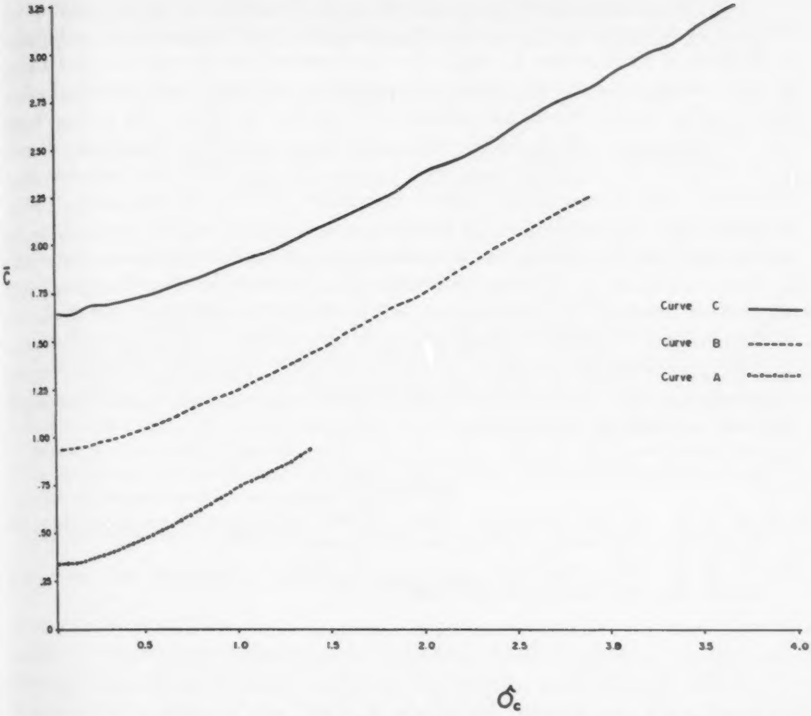

FIG. 6. Relationship between mean catch ($\bar{c}$) and standard deviation of catch (σ_c) in completely stabilized fisheries for 200 year simulations for curves A, B, and C for which environmental effects at various levels were simulated by scaled random normal deviates used in the method of Ricker (1958).

with the particular $\bar{c}$ and σ_c considered. Having an estimate of a, solving for w_m the expression

$$-aw_m e^{-a(1-w_m)} + e^{a(1-w_m)} - 1 = 0,$$

and comparing this value to the actual escapement, provides the check. If they are identical, it may be concluded that m.e.c. is being obtained. Harvesting at levels other than m.e.c. results in a decrease of $\bar{c}$, which will cause the theoretical and actual escapement values to differ.

DISCUSSION

The superimposition of simulated long-term environmental effects on stock recruit systems does not alter the general conclusions of Ricker (1958). Environmental variability, whether of a random or regular nature, results in benefits from complete stabilization of escapement and, the greater the variability, the greater the benefits.

This conclusion in large part hinges on the validity of the assumptions implicit in the procedure of simulating environmental effects by using multipliers or divisors of production. It might be more reasonable to suppose that a stock at high density would be more susceptible to harmful environmental effects (such as low water levels for salmon). In such a situation the scaling factor for environmental effects might be made larger at higher densities. A great many techniques might be contrived to approximate whatever was envisaged. However, there is relatively little information on the mechanisms by which environmental factors influence production at various stock levels. It is also not known that the values of relevant environmental variables are distributed in the special pattern of Ricker's system (it is to be noted that the distribution of random normal deviates augmented by one as multipliers and divisors is not log normal). Further understanding of stock recruit systems, as influenced by environmental fluctuations, probably depends primarily on more detailed knowledge of the mechanisms by which environmental factors influence production at various stock sizes.

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Reaction of Free Fatty Acids with Protein in Cod Muscle Frozen and Stored at –29 C after Aging in Ice

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Freezing and holding cod muscle in the frozen state favored the association process that involves protein–free fatty acid (FFA) complex formation and begins during aging in ice. Changes in protein extractability, in ultracentrifugal patterns of protein extracted, and in phase contrast micrographs of inextractable muscle fragments were followed in muscle that had been aged in ice to produce various contents of FFA and then frozen and held at –29 C. After 11 months, these changes, which took place largely during the first week of storage, were comparable with those that occur when the FFA are formed during frozen storage. The results were consistent with a reaction rate that was greater at –29 C than at temperatures a few degrees above 0 C.

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INTRODUCTION

TWO HYPOTHESES have been proposed to explain decrease in protein extractability in frozen-stored fish muscle (Connell, 1964). Protein is thought to be damaged as a consequence of continued exposure to concentrated solutes in the frozen-stored muscle in the "salt denaturation" theory, or as a consequence of lipid degradation in the lipid hydrolysis theory. In the latter case, protein is protected by lipids against denaturation and protection is lost when lipids are hydrolyzed; or, alternatively, free fatty acids (FFA), products of lipid hydrolysis, denature the protein. It is also possible that reaction with oxidized lipids may be involved in this type of protein denaturation.

Model systems studies in which long-chain fatty acids were shown to decrease protein extractability (King et al., 1962; Anderson et al., 1963; Anderson and Steinberg, 1964) have renewed interest in the lipid hydrolysis theory. It was postulated (Anderson et al., 1965) that interaction of contractile protein with FFA in the liquid phase of frozen muscle is the cause of decreased protein extractability.

The last hypothesis seemed to be a difficult one to test in frozen-stored muscle, because the effects on muscle proteins of progressive lipid hydrolysis with liberation of FFA could not be determined independent of the effects of exposure to the changed environment brought about by freezing (van den Berg, 1961).

We (Anderson and Ravesi, 1968) have established that: (1) decrease in protein extractability in cod muscle aged in ice is due to an association process

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involving contractile protein–FFA complex formation; and (2) decrease in protein extractability in this muscle is small even in the presence of large amounts of FFA, because the complexes are not stable and dissociation occurs during extraction. We proposed that acceleration of the reaction and stabilization of the reaction products in the frozen state may account for the larger decreases in protein extractability that have been observed in frozen-stored muscle of similar FFA content.

In this study we have dissected what we believe to be the normal process of deterioration induced in frozen storage; that is, we have separated the effects of holding in the frozen state from the effect of FFA liberation by producing muscle of various FFA contents by aging it in ice for different lengths of time, and then aging it further in the frozen state at a temperature low enough so that little additional lipid hydrolysis would be expected. In this way, we could determine the roles freezing and holding in the frozen state play in bringing together the reactants, FFA and contractile protein, through following changes in: (1) protein extractability; (2) composition of protein extracted as seen in ultracentrifugal patterns of extracts; and (3) phase contrast micrographs of inextractable muscle fragments.

EXPERIMENTAL

Muscle tissue for extraction studies consisted of samples of myotomes dissected (Anderson et al., 1965) from gutted cod, *Gadus morhua*, weighing 15–25 lb each. This lot of fish, which was aged in ice in a room held at 4 C for from 1 to 35 days, was sampled at 10 different intervals during this period. Myotomes dissected from another lot of cod stored in ice for 25, 33, and 39 days were used to study the rate of the reaction at −29 C and to obtain ultracentrifugal patterns of protein extracts from muscle unfrozen and frozen after prolonged aging in ice. For each storage interval, myotomes were cut from six fish, pooled, and mixed. From each pool, 10-g samples were weighed in disposable aluminum dishes, packaged in threes in Cryovac[1] pouches, and vacuum-sealed.

Tissue samples were frozen by placing the packages in single layers on metal shelves in a room at −29 C. Samples used for determining the effect of freezing were left for 2 hr in the room cooled to −29 C. They were then thawed just to the softening point in a room at about 23 C and used immediately for extraction studies. Samples used for determining the effect of holding in the frozen state were placed in cartons 24 hr after freezing and stored at −29 C. Preliminary studies (unpublished data) had shown that myotomes from fresh cod could be stored in this fashion for several months at −29 C without significant change in extractable protein content.

Samples of myotomes aged for 1–35 days were examined before freezing, after 2 hr at −29 C, and after 11 months at −29 C. Samples aged for 25, 33, and 39 days in ice were examined after being frozen for 2, 24, and 48 (except 39-day sample) hr and examined periodically thereafter for about 10 weeks to determine the rate of change in protein extractability at −29 C. Protein and lipid extracts of myotomes were prepared in triplicate essentially as has been previously described (Anderson and Ravesi, 1968). For determination of extractable protein content, muscle-extractant homogenates were prepared using a 90-sec blending procedure (Anderson et al., 1963). Extractants for water soluble, or sarcoplasmic, protein (0.05 ionic phosphate buffer, *p*H 7.5) and for total extractable protein (0.5 ionic buffered KCl) were those described by Connell (1958). The tissue to extractant ratio was 10:90 for low ionic strength extracts and 10:390 for 0.5-ionic-strength extracts. Muscle tissue samples were prepared and extracted in a cold laboratory at 1 C.

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[1] The use of trade names is merely to facilitate descriptions; no endorsement is implied.

For determination of extractable protein, a portion of muscle-extractant homogenate was centrifuged at 1100 g for 20 min in a refrigerated centrifuge at 1 C immediately after preparation. The protein content of the supernatant fluids was assayed either by a biuret method (Layne, 1957) or an automated method (Failing et al., 1960).

To monitor changes in composition of protein extracts, sedimentation patterns were obtained with a Spinco Model E analytical ultracentrifuge using schlieren optics. Ultracentrifugation was carried out at a constant temperature of 1 C and a rotor speed of 50,740 rpm. Single sector cells with an optical light path of 12 mm, unless otherwise stated, were used.

To follow changes in structure of inextractable material, wet preparations of residues of centrifuged muscle-extractant homogenates were examined by phase contrast microscopy, and photomicrographs were taken as previously described (Anderson and Ravesi, 1968).

Lipid extracts of myotome samples were prepared by the method of Folch et al. (1957). Lipids associated with low ionic strength protein extracts were extracted using the technique of Bligh and Dyer (1959), treating the extract as water in this procedure.

Phospholipid-free fractions of total lipid extracts were prepared by the method of Antonis (1965). Free fatty acid contents of the phospholipid-free extracts were estimated using a modification of the colorimetric technique of Novàk (1965), in which heptane was added to the chloroform extracts to give the required chloroform–heptane ratio of the procedure.

EFFECTS OF FREEZING

PROTEIN EXTRACTABILITY

Freezing, even for a period somewhat less than 2 hr, caused decreases in extractable protein in addition to the decreases that had already occurred during aging in ice (Fig. 1). In general, the decreases were larger in muscle that had been aged in ice 19 or more days than in muscle aged for shorter periods; they were greatest in 35-day muscle, in which protein extractable dropped from 65 to 42%.

ULTRACENTRIFUGAL PATTERNS

The effects of freezing the muscle for a short time were perceptible in the sedimentation patterns of readily extractable protein extracts of muscle that had been stored in ice only 1 day (Fig. 2). When compared with the pattern obtained before the muscle iced 1 day was frozen (Fig. 2A1–3), the pattern after freezing of the muscle (Fig. 2B1–3) showed a smaller, faster sedimenting peak II (actomyosin in the system used) with less skew in the leading edge, a smaller peak IIIA, a sharper turnup of the pattern close to the pellet early in the run and a larger pellet. With longer iced-storage of the muscle, these changes brought about by brief freezing became more noticeable. Beginning with 15-day-iced muscle, the area under the turnup was smaller in patterns obtained before the muscle was frozen (not illustrated). This trend became more noticeable in 19- and 22-day-iced muscle and is seen in Fig. 2C(1–3), showing extract patterns of 22-day-iced muscle before and after freezing of the muscle. The smaller and faster peak II in the pattern from the frozen muscle is evident.

In patterns of muscle iced for prolonged periods before freezing, buildup of rapidly sedimenting components during centrifugation was greater than in the 22-day pattern. There was a small shoulder on the turnup in the pattern for 25-day muscle (Fig. 3A1–3, upper pattern). The shoulder was absent in the pattern for unfrozen muscle (Fig. 3A1–3, lower pattern). Patterns of extracts of 33-day muscle (Fig. 3B1–3) showed a fast peak close to the pellet in extracts of both unfrozen and frozen muscle. Comparison of the patterns for 39-day muscle (Fig. 3C1–3) shows clearly that the large decrease in extractable protein after freezing of the muscle was at the expense of peak II and more rapidly sedimenting components.

No attempts were made to treat the ultracentrifugal data other than grossly because of the very high degree of polydispersity of the components of interest. Rapid changes in concentration, particularly in the area close to the pellet where buildup of rapidly sedimenting material occurred, would preclude the calculation of apparent sedimentation rates. Neither was the effect of freezing on peak III (myosin) evaluated.

MICROSCOPIC EXAMINATION OF RESIDUES OF MUSCLE-EXTRACTANT HOMOGENATES

Greater resistance to homogenization after freezing was indicated by larger fragment size in residues of muscle frozen after aging in ice for 25, 33, and 39 days (Fig. 4) than in the residues of unfrozen muscle. For example, fragments from unfrozen 25-day (Fig. 4A) and 33-day (Fig. 4D) muscle were small bundles of myofibrils; fragments from 25-day frozen (Fig. 4B, C) were large bundles of myofibrils; and from 33-day (Fig. 4E) and 39-day (Fig. 4F) largely chopped fibers.

EFFECT OF FROZEN STORAGE

For muscle iced for 1–12 days, total FFA contents averaged about 34 mg/100-g myotomes higher after 11 months at –29 C than before freezing (Fig. 5). For muscle iced 15–35 days, the FFA contents tended to be lower after frozen storage than before freezing.

FFA contents of sarcoplasmic protein extracts were lower after frozen storage than before (Fig. 5). Correction for variability in protein content of these extracts (Table 1) shows that, for muscle iced 12–35 days before frozen storage, the recovery level after freezing was roughly one-half that before freezing.

PROTEIN EXTRACTABILITY

Decreases in readily extractable protein were much greater after 11 months than after 2 hr at –29 C (Fig. 1). In general, percentage of protein extractable decreased with increasing storage in ice. It was 25% in 35-day muscle.

ULTRACENTRIFUGAL PATTERNS

Decreases in peak II (actomyosin) were much greater in ultracentrifugal patterns for muscle stored for 11 months at –29 C (Fig. 6) than for similar muscle after freezing for less than 2 hr (Fig. 2, 3). With frozen storage, the

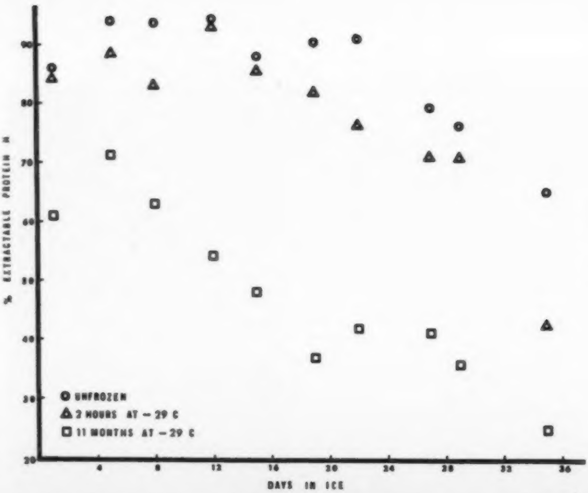

FIG. 1. Effects of freezing and holding at −29 C for 11 months on the extractable protein content of cod muscle aged in ice for 10 periods.

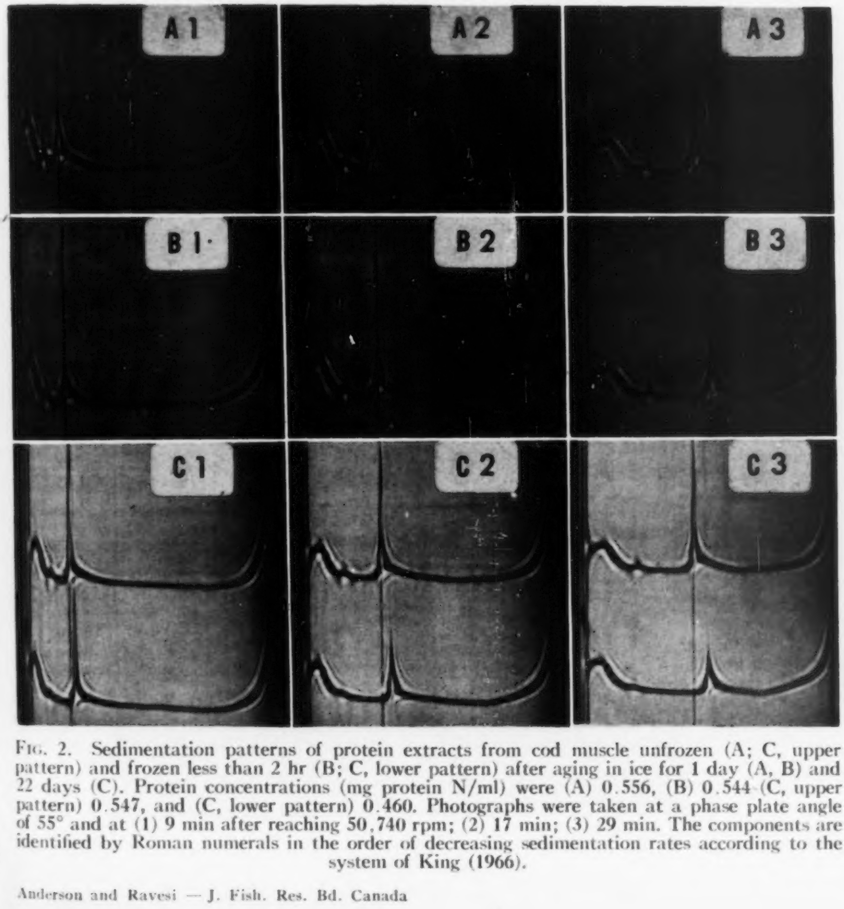

FIG. 2. Sedimentation patterns of protein extracts from cod muscle unfrozen (A; C, upper pattern) and frozen less than 2 hr (B; C, lower pattern) after aging in ice for 1 day (A, B) and 22 days (C). Protein concentrations (mg protein N/ml) were (A) 0.556, (B) 0.544 (C, upper pattern) 0.547, and (C, lower pattern) 0.460. Photographs were taken at a phase plate angle of 55° and at (1) 9 min after reaching 50,740 rpm; (2) 17 min; (3) 29 min. The components are identified by Roman numerals in the order of decreasing sedimentation rates according to the system of King (1966).

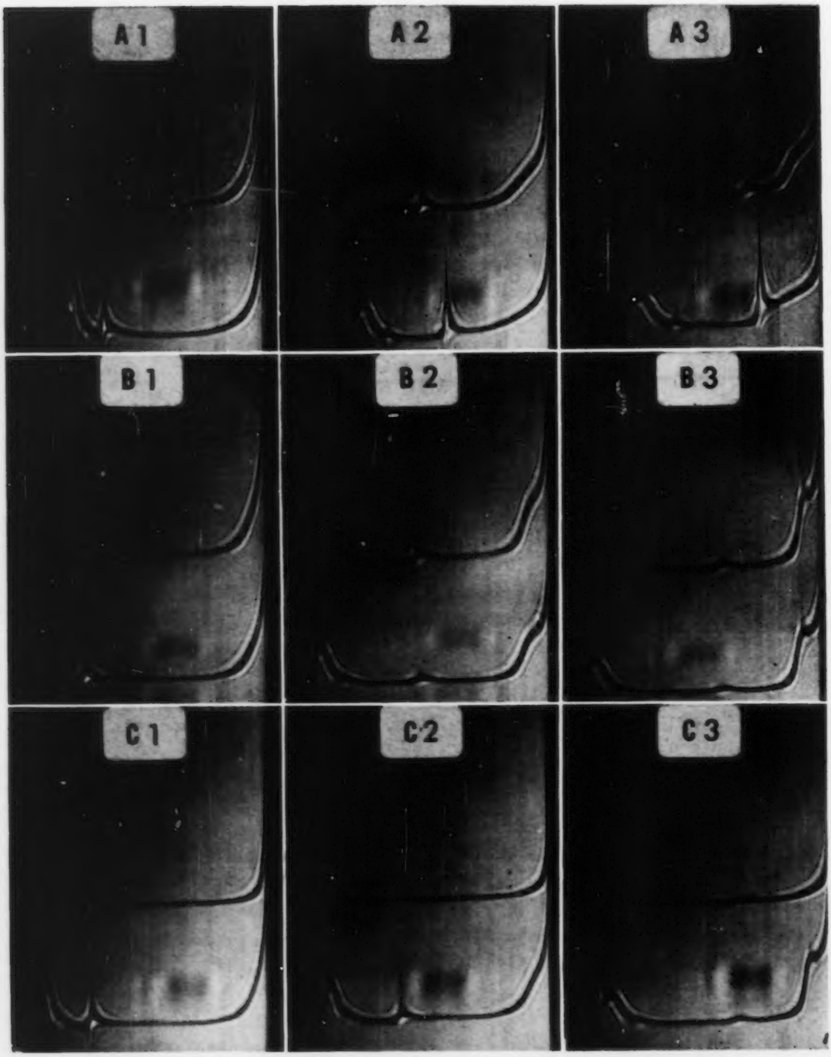

FIG. 3. Sedimentation patterns of protein extracts from cod muscle unfrozen (lower patterns) and frozen less than 2 hr (upper patterns) after aging in ice for 25 days (A); 33 days (B); 39 days (C). Protein concentrations (mg protein N/ml) were (A) 0.526, (B) 0.412, and (C) 0.387 for lower patterns and (A) 0.428, (B) 0.296, and (C) 0.232 for upper patterns. Photographs were taken at a phase plate angle of 55° and after reaching 50,740 rpm: (A1) 4 min, (A2) 16 min, (A3) 24 min, (B1) 5 min, (B2) 13 min, (B3) 17 min, (C1) 4 min, (C2) 8 min, and (C3) 16 min.

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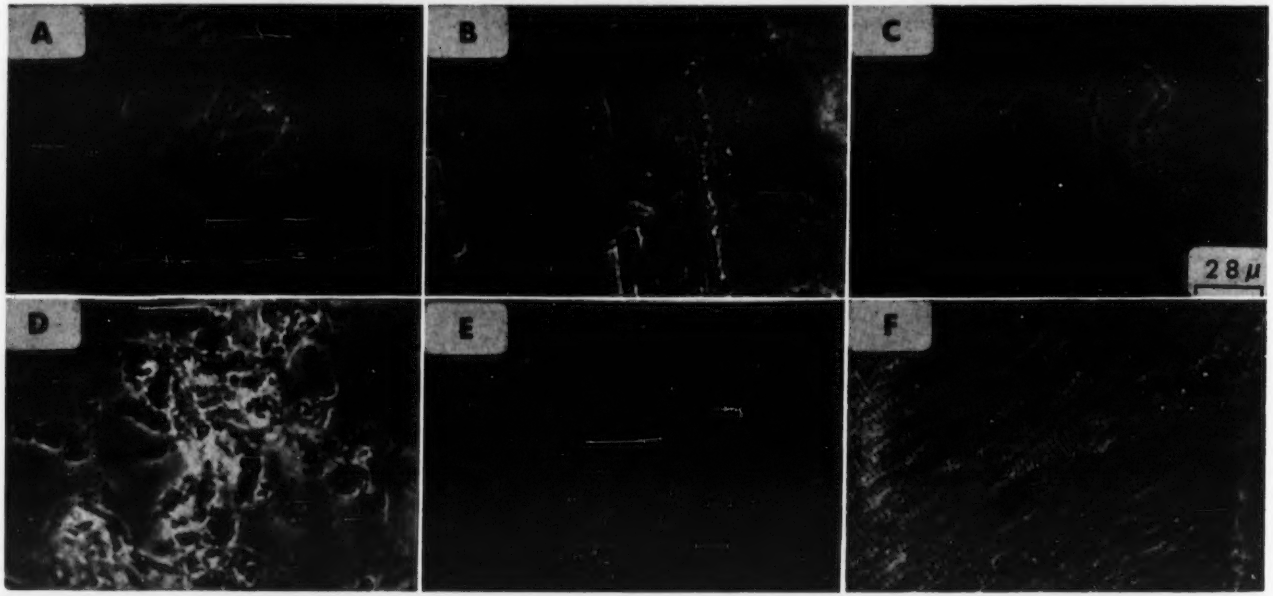

FIG. 4. Phase contrast micrographs showing the effect of brief freezing of the muscle on size of fragments in residues of homogenates prepared from muscle unfrozen (A, D) and frozen briefly (B, C, E, F) after aging in ice for 25 days (A, B, C), 33 days (D, E), and 39 days (F). Magnification for all photographs was as indicated in C.

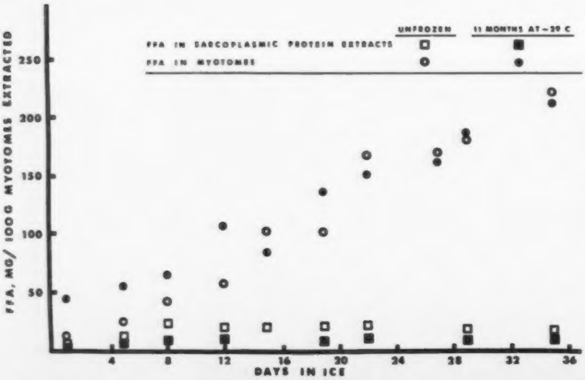

FIG. 5. Change in FFA contents of cod myotomes and their sarcoplasmic protein extracts after the myotomes from fish aged in ice for 10 periods were stored for 11 months at −29 C.

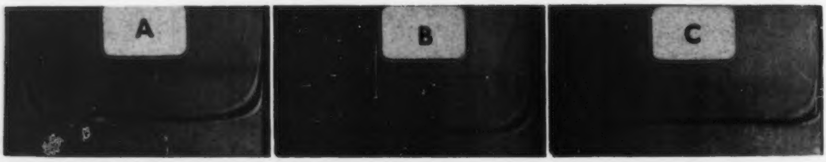

FIG. 6. Sedimentation patterns of protein extracts from cod muscle stored at −29 C for 11 months after aging in ice for (A) 1 day, (B) 19 days, and (C) 27 days. Protein concentrations (mg protein N/ml) were (A) 0.394, (B) 0.222, and (C) 0.251. Photographs were taken 9 min after reaching 50,740 rpm. The light path of cell used for A2 was 18 mm.

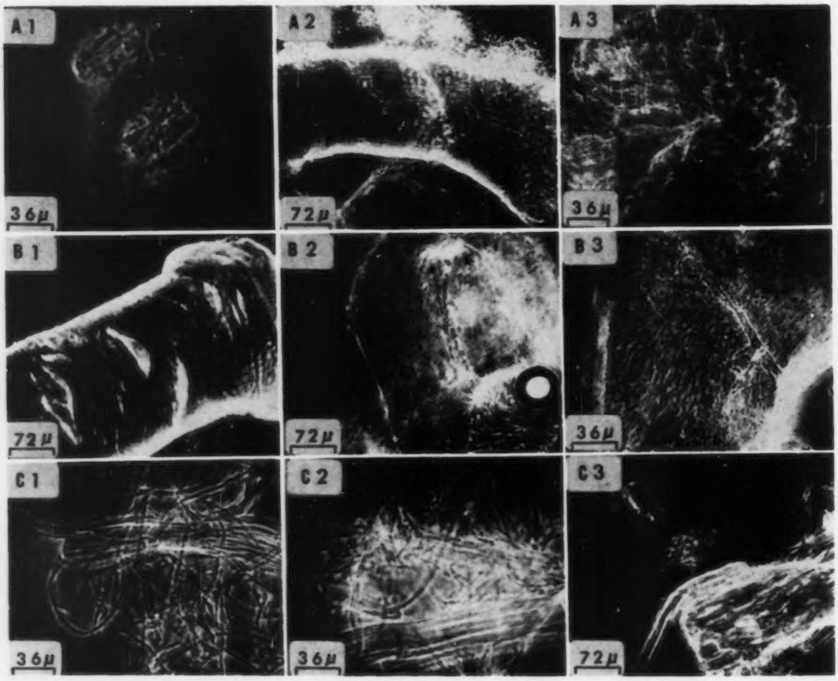

FIG. 7. Phase contrast micrographs of fragments in residues from homogenates of muscle stored at −29 C for 11 months. Homogenates were prepared in 0.5 ionic extractant from muscle iced (A1) 1 day, (A2) 12 days, and (A3) 27 days; in 0.5 ionic extractant from muscle iced 35 days (B); and in 0.05 ionic extractant from muscle iced (C1) 27 days, (C2) 29 days, (C3) 35 days.

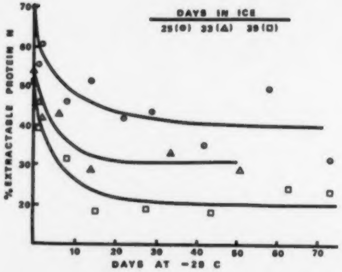

FIG. 8. Changes in protein content of extracts prepared from cod muscle stored at −29 C for various periods after aging in ice for 3 periods.

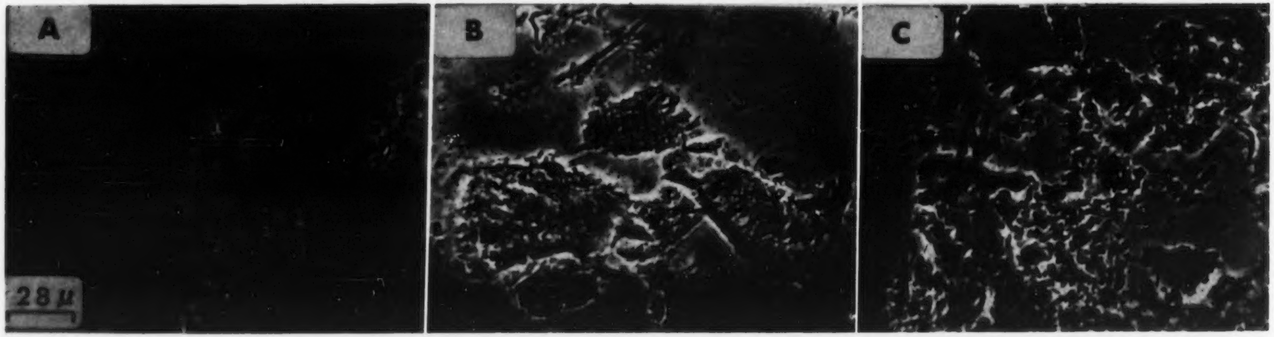

FIG. 9. Phase contrast micrographs of residues of homogenates prepared in 0.5 ionic extractant from muscle frozen (A) 8 weeks, (B) 2 weeks, (C) 1 week. Muscle had been aged in ice before freezing: (A) 25 days; (B) 33 days; and (C) 39 days.

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size of this peak decreased progressively according to duration of storage in ice, from small for muscle iced 1 day (Fig. 6A) to negligible for 19 days (Fig. 6B) and absent for that iced 27 days (Fig. 6C).

Photomicrographs of Residues of Muscle-Extractant Homogenates

The residues of muscle aged in ice for 25 or more days showed significant cohesion of myofibrils after the muscle was stored for only 2 hr at –29 C; residues of muscle aged for shorter periods in ice showed cohesion after the muscle was stored for 11 months at –29 C (Fig. 7). Predominant fragment size in residues increased progressively according to duration of storage in ice, from small bundles for muscle iced 1 day (Fig. 7A1) to much larger fragments, many of which were of full fiber width, for muscle iced 12 days (Fig. 7A2), and to mostly transversely severed fibers for muscle iced beyond 19 days. There were, however, many small fragments together with much material having a smudged appearance (Fig. 7A3) for muscle aged up to 29 days. In 35-day residues, fragments were almost all severed fibers, about 2–3 times as long as thick (Fig. 7B1). In many cases, these fragments and some from muscle iced for shorter periods were severed at about the same distance from what appeared to be points of joining of the transverse tubules (T System) with the sarcolemma (Fig. 7B2, 7B3).

When muscle stored 11 months at –29 C was homogenized in 0.05 ionic extractant, transversely severed fibers were predominant for muscle iced 23–35 days. Smaller fragments in these residues, in contrast to those in 0.5 ionic residues, appeared to have rather regular band patterns (Fig. 7C1–3).

RATE OF REACTION

Protein Extractability

In muscle sampled after prolonged storage in ice, extractable protein decreased rapidly during the first few days of storage at –29 C (Fig. 8). After this, the rate of decrease was slower than before; and after about 2 weeks, there was little further change. Percentages at which protein extractability plateaued were related to storage time in ice: 45 in 25-day muscle; 30 in 33-day; and 20 in 39-day, a value close to that generally accepted as minimum extractability in cod muscle after prolonged aging at –14 C (Connell, 1964). The data have been treated grossly here and are reported graphically, since the methods for assessing protein extractability are not independent of the dissociation that occurs during extraction (Ravesi and Anderson, 1969).

Microscopic Examination of Residues of Muscle-Extractant Homogenates

Size of typical fragments in residues of muscle frozen after prolonged aging in ice increased very early in the storage period. Transversely severed fibers were predominant in residues of 25-day muscle frozen 1 week and in

33- and 39-day muscle frozen 24 hr. Many small frgaments were also seen, however, and continued to be seen in residues of muscle stored for longer periods (Fig. 9).

DISCUSSION

Effects of Freezing

Although decreases in readily extractable protein after brief freezing of muscle aged in ice were small, they were larger, in general, with increasing storage of the muscle in ice and with accompanying increased FFA content of the muscle. They were not so large as in muscle in which similar concentrations of FFA had been liberated as a result of lipid hydrolysis during frozen storage (Dyer and Fraser, 1959; Olley et al., 1962). The observed decreases probably do not reflect the true extent of in situ changes due to brief freezing, since it is likely that dissociation occurred during homogenization as it did when muscle that had simply been aged in ice was homogenized (Anderson and Ravesi, 1968).

Ultracentrifugal patterns of protein extractable after brief freezing of muscle aged in ice show that decreases in protein extractability occurred at the expense of actomyosin, the component designated IIIA, and heavier components formed in muscle that had undergone prolong storage in ice. This is evidence that freezing brought about a greater degree of the same kinds of changes involving protein–FFA interaction that were shown to take place in muscle aged in ice (Anderson and Ravesi, 1968). Increased cohesion of myofibrils in fragments found in homogenates of frozen muscle is also evidence for continuation of the association process noted in this muscle before freezing.

Changes observed after freezing might be greater if muscle aged in ice were held in the frozen state, and the results of the frozen storage study bear this out.

Effect of Frozen Storage

Comparison of the extractable protein contents of muscle held in the frozen state for 11 months after aging in ice for various periods (Fig. 1) with the FFA contents of this muscle (Fig. 5) shows that decreases in percent protein extractable were close to those reported for muscle in which similar concentrations had been developed during frozen storage (Dyer and Fraser, 1959; Olley et al., 1962). Moreover, these decreases are related largely to the FFA that had been formed during aging in ice, since changes in FFA contents of this muscle during the 11 months at -29 C were small. It should be emphasized that percent protein extractable, even in the same sample of muscle, would not be expected to be the same with the techniques used in this study as with the methods used by the other two groups of investigators, since they used extractants of higher ionic strength and different blending times. The salt content of extractant and the blending time have been shown to influence protein extractability in frozen-stored muscle (Ravesi and Anderson, 1969). Also, Dyer and Fraser express their results in terms of extractable actomyosin. Differences in extraction technique may also explain the differences between results reported here, e.g., decreased protein extractability after storage at -29 C of muscle of low FFA content, and those of Love (1962), who reported little change in protein extractability after $1\frac{1}{2}$ years at that temperature. Extraction in 0.5 ionic instead of 5% salt solution favors the detection of more subtle changes (Ravesi and Anderson, 1969).

Ultracentrifugal patterns of protein extracts show that decreases in readily extractable protein after frozen storage are largely at the expense of actomyosin and, in muscle of high FFA content, heavier components formed during prolonged aging in ice. Changes in myosin would not have been easily assessed in the extracts studied because of the number of components extracted, especially from muscle that had been aged 25 days and more in ice. There were other small decreases that should be mentioned, e.g., in the component designated IIIA and in sarcoplasmic protein (Table 1). This latter decrease would be expected when, as microscopic examination of inextractable material showed, many muscle fibers were fragmented only transversely (Fig. 7).

TABLE 1. Protein contents of 0.05 ionic extracts of cod muscle and ratios of FFA to protein extracted before and after storage for 11 months at -29 C after aging in ice.

Days in ice:	1	5	8	12	19	22	29	35
Protein N before storage (*g/100 g muscle extracted*)	0.526	0.612	0.580	0.435	0.466	0.457	0.430	0.400
FFA (*mg*)/g protein N before storage	7.6	20.6	42.1	45.5	44.8	44.4	39.8	39.8
Protein N after storage (*g/100 g muscle extracted*)	0.516	0.567	0.574	0.479	0.457	0.419	0.408	0.390
FFA (*mg*)/g protein N after storage	7.5	10.2	12.7	20.2	18.0	20.8	18.9	22.0

Changes seen in the microstructure of inextractable muscle fragments, especially those prepared from muscle stored 11 months, are interesting on two accounts: First, areas of the muscle fiber similar to those seen in Fig. 7 (B2, 3) appear to resist fragmentation. The T system is known to be relatively high in lipid content. During aging of the muscle in ice, diffusion of FFA into the myofibrils adjacent to the T system after release from foci of high lipid content is one possible explanation for the prevalence of fragments severed at about the same distance from what appear to be parts of the T system. Second, distortion of the banding pattern in residues in which many of the fragments have a smudged appearance suggests in situ changes in microstructure. Failure to find much gross evidence for distortion of the banding pattern in residues of muscle homogenized in low ionic strength buffer (i.e., in the absence of an extractant for contractile protein) argues against in situ change. Smudged appearance could be due simply to incomplete dissolution of protein from its normal position in the microstructure or to changes in the state of aggregation of complexed protein on exposure to the extractant. The presence of aggregated protein in extracts does not resolve this problem. The observed aggregates may not exist as such in the muscle.

Rate of Reaction

Taken together, the results of the extraction and microscopic studies of muscle stored for short periods at –29 C after prolonged aging in ice showed that the association process that begins during aging of the muscle in ice proceeded very rapidly during the first few days of subsequent storage at –29 C. The rate was slower for the next 10 days or so and became very slow after about 2 weeks. The extent of the association was related to the iced-storage age of the muscle. The extractable protein contents of 33- and 39-day muscle after about 2 weeks' storage at –29 C (Fig. 8), when compared with that of 35-day-iced muscle held at –29 C for 11 months (Fig. 1), suggest that decrease in protein extractability observed in the muscle stored 11 months occurred largely during the first few weeks of storage. A similar comparison can be made between 22- and 27-day-iced muscle stored 11 months at –29 C and 25-day-iced muscle stored a few weeks.

Decreases in protein extractability occurred more rapidly in the frozen muscle. Comparison of the extractable protein contents of 25- and 33-day-iced muscle before freezing (Fig. 8) shows that during the 8 days' additional storage in ice, the protein extractable decreased from 70 to 54%. Similarly, comparison of the protein contents of 33- and 39-day-iced muscle shows that during 6 days' additional storage of 33-day muscle, the protein extractable decreased from 54 to 51%. Decreases in percent protein readily extractable after 1 and 2 days at –29 C is roughly equivalent in 25-day muscle and larger in 33-day muscle than decreases that would occur in 8 and 6 days' additional storage in ice of 25- and 33-day muscle. During iced storage, moreover, the FFA of the muscle would be increasing; in muscle held at –29 C, there would be very little FFA formed. These results are interpreted as an enhancement of the rate of the association process in muscle held in the frozen state.

Reactions rate in aqueous systems that are greater in the frozen state than at 0 C and somewhat above are not new. Grant and his associates (Grant and Alburn, 1967; Grant, 1966) reported that certain reactions proceed more rapidly in ice than in liquid water, and more recently Buttkus (1967) found that reaction of malonaldehyde with the Σ-amino groups of myosin proceeds more rapidly at +20 C and –20 C than at 0 C.

General

A number of investigators have reported that freshness or staleness of the muscle before freezing influences the stability of muscle protein during subsequent frozen storage. Their results, reviewed by Love et al. (1965), are not in agreement. Love et al. (1965) studied the effects of freezing and of storing at -14 C on muscle aged in ice for various periods. Their findings are similar to those reported here on two accounts: first, that loss in soluble protein during storage in ice increases with increased iced-storage age of the muscle; and, second, that loss in soluble protein after frozen storage increases in general with duration of storage in ice.

In interpreting their results, Love et al. suggest that what happens during aging of the muscle in ice is not related to the changes that take place during storage at -14 C. Rather, they say, "the mechanism responsible for toughening during cold storage of cod fillets had been triggered off by the freezing and thawing." Interpreted in the light of the findings of the present study, their results are further evidence that reaction of muscle protein with FFA begins during aging in ice and is favored during frozen storage. This is not to say that the studies are similar in all respects. In the study reported here, the FFA involved was largely formed during aging in ice; in the study by Love et al., considerable additional FFA would be expected to be formed during storage at -14 C, and decreases in soluble protein would be due to reaction involving FFA liberated at -14 C, as well as FFA formed prior to freezing,

The results reported here should be of particular interest to the fishing industry since they affirm the necessity of using only prime quality fish for freezing if maximum frozen storage life is to be expected.

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Effects of Delayed Mating on the Reproduction of King Crab, *Paralithodes camtschatica*

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MCMULLEN, JOHN C. 1969. Effects of delayed mating on the reproduction of king crab, *Paralithodes camtschatica*. J. Fish. Res. Bd. Canada 26: 2737–2740.

Mature female king crab, *Paralithodes camtschatica* (Tilesius), molt annually, at which time each is normally attended by a male for mating purposes. This study considered how mating and the production of fertilized eggs might be affected if female king crab were forced to await partners after molting, a situation that has been observed at Kodiak Island.

Prior to studying delayed mating, a carapace length–egg clutch volume relation was developed so partial egg clutches that might be produced could be objectively evaluated. The regression equation calculated is $Y = -248.55 + 2.69\ X$, where carapace length was measured in millimeters and clutch volume in milliliters. The coefficient of determination (r^2) for this regression equation is .899.

The study of delayed mating was conducted with 76 female king crab, which were allowed to mate on one of 2–15 days after molting. All females that mated within 9 days after molting produced fertilized egg clutches. Nine of 24 females allowed to mate 10–13 days following ecdysis, and 10 females mated after 13 days, produced no egg clutches.

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INTRODUCTION

ALONG THE COAST of Alaska, mature female king crab, *Paralithodes camtschatica* (Tilesius), molt during the spring. Normally each molting female is accompanied by a male king crab that mates the female immediately after her molt. The mating act is accomplished by the deposition of spermatophore material around the female's oviducts. After mating, the female's ripened eggs are extruded, fertilized, and attached to her pleopods.

The presence of an egg clutch may not indicate a female king crab was mated at her last molt. McMullen and Yoshihara (1968) observed that apparently unmated females may retain their ripe eggs in their ovaries, where they are resorbed, or the females may extrude and carry the unfertilized eggs until those eggs decay.

King crab mating behavior has previously been examined, but observations concerning the results of delayed mating have varied. Kurata (1961) stated that female king crab would not spawn normally if mated later than 5 days after ecdysis. Wallace et al. (1958) stated that, "Females which were placed in traps and allowed to moult in the absence of males did not extrude their eggs within an observation period of three weeks, until they were allowed to mate."

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This study was designed to investigate the relation between molting and mating for king crab, particularly the period after molting in which females could be mated and produce fertilized egg clutches and the effect of delayed mating on size of egg clutches.

MATERIALS AND METHODS

King crab for this study were collected during April and May, 1968. Females for delayed mating were captured in premolt condition while being grasped by males, ensuring their suitability for study use.

Carapace Length–Egg Clutch Volume Relation

A group of postmolt females, captured at Kodiak Island, Alaska, with apparent full clutches of new eggs, was used to determine the relation between carapace length in millimeters and clutch size milliliters. This relation was required to make possible a quantitative estimate of partial egg clutch sizes that might result from delayed mating. Bright et al. (1960) examined egg production of king crab in Kachemak Bay, Alaska, but their results are not directly applicable to this study because the eggs probably had passed through the cleavage stages and had enlarged with embryo growth.

Egg clutch volume was determined by the displacement method. After removal from the females' pleopods with a scalpel, the eggs were rolled in paper towelling to remove excess water, then measured in sea water. Measurement by volumetric displacement is valid, for Haynes (1968) showed there is no significant change in egg size for different size female king crabs.

Delayed Mating

A second group of females was utilized in the delayed mating study. They molted in captivity in the absence of males, after which subgroups were placed with mature males on a consecutive series of 2–15 days after ecdysis. Subgroups ranged from 5 to 8 females. Members of a subgroup were placed with males at a constant elapsed time after ecdysis, but on different calendar dates.

Male king crab used as brood stock were captured in natural mating areas. In captivity, they were not allowed to mate with two females consecutively, but were rested. They were generally used twice, then replaced. An extra male was always placed in each mating box to negate the possibility of a sexually inactive male.

All king crab were held in three floating wood-slat boxes (244 × 122 × 122 cm). Reserve males were held in a compartment of one box, the other half of which was occupied by premolt females and mated females with eggs. Study results could not be affected by those particular individuals being in close proximity of each other. A second box was divided into two mating chambers, and the two compartments of the third box contained mated females and those to be mated.

After being placed with males the experimental females were examined for the presence of spermatophore material around their oviducts as evidence of mating.

After being given an opportunity to mate, the female crab were held for 2–3 weeks, then released. Eggs were removed from each egg-bearing female 7–10 days after mating. Egg clutches were stripped from the pleopods and measured volumetrically without preservation. Those eggs to be examined microscopically for fertilization were fixed in Bouin's solution and preserved in alcohol.

RESULTS AND DISCUSSION

Carapace Length–Egg Clutch Volume

Egg clutches of 54 new-shell females comprising the control group ranged from 44 to 210 ml and their carapace lengths from 103 to 168 mm. The relation of carapace length–egg clutch volume appeared to be somewhat curvilinear, but a regression line has been fitted for the purpose of this study (Fig. 1). The calculated regression equation for king crab female carapace length–egg clutch volume is $Y = -248.55 + 2.69\ X$, where Y represents milliliters of eggs expected to be carried by a given size (X) female mated normally. The coefficient of the determination (r^2) for this regression equation is .899.

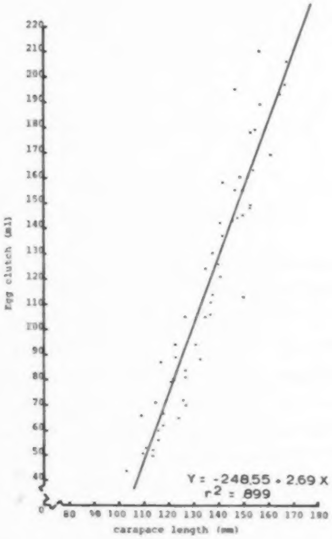

FIG. 1. Relation for carapace lengths–egg clutch volumes of new-shell female king crab caught at Kodiak Island, spring of 1968.

DELAYED MATING

Female king crab that were allowed to mate through 9 days after ecdysis produced egg clutches (Table 1). One female of the subgroup mated 15 days after ecdysis had a trace of spermatophore material at her oviducts; no spermatophore material was evident on the other unproductive females.

Female king crab delayed in mating produced apparent full egg clutches. Since no partial clutches were extruded, the carapace length–clutch volume relation was not required for evaluation.

Eggs of all clutches produced were classified as fertile after microscopic examination revealed that they were in advanced cleavage stages.

Results of this study differ from those obtained by other authors, but the differences are partially explainable. All the female king crab in this study allowed to mate between 2 and 9 days following ecdysis produced clutches of fertilized eggs. About 56% of 18 females allowed to mate at 10–13 days after ecdysis produced fertilized clutches, but none of 10 females allowed to mate at 14–15 days did so. As all egg clutches appeared to be full, I did not use a calculated carapace length–egg clutch volume as a standard.

TABLE 1. Effects of delayed mating on the production of fertilized egg clutches by king crab.

Females placed with males		Females producing egg clutches	
Days after ecdysis	No. (n_1)	No. (n_2)	% of group
2	6	6	100
3	6	6	100
4	5	5	100
5	5	5	100
6	5	5	100
7	5	5	100
8	5	5	100
9	5	5	100
10	6	5	83
11	8	5	63
12	5	2	40
13	5	3	60
14	5	0	0
15	5	0	0

Results of this study also indicate that female king crab delayed in mating more than 13 days after ecdysis fail to successfully mate and produce clutches of fertilized eggs because they are not selected by males. This is indicated by the absence of spermatophore material around the oviducts of all but one female not producing egg clutches after placement with males. The trace of spermatophore material may have been deposited by a male that responded to the female's aggressiveness. I have observed aggressive behavior when females were placed in aquaria with males 11–13 days after molting.

Females of this study not mated successfully did not extrude eggs, but individuals of this species are known to do so. Perhaps the female king crab that Wallace et al. (1958) placed with males 3 weeks after ecdysis produced and carried unfertilized eggs.

King crab females normally mate immediately after molting. However, they may successfully mate and produce normal clutches of fertilized eggs 9–13 days after ecdysis if obliged to wait for polygamous males present in inadequate numbers.

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NOTES

Allocation of Weight and Length Measurements for Estimation of the Average Weight of Juvenile Sockeye Salmon[1]

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MATHISEN, OLE A. 1969. Allocation of weight and length measurements for estimation of the average weight of juvenile sockeye salmon. J. Fish. Res. Bd. Canada 26: 2741–2746.

The regression of weight on length of juvenile sockeye salmon was utilized to determine the optimal division of weight and length measurements in order to obtain minimum variance of the average weight estimate with a fixed expenditure of time to observations.

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THERE ARE SEVERAL REASONS for estimating weight of juvenile sockeye salmon. One is related to the trophic dynamics of the nursery lakes where the relationship between primary and secondary producers and fish production must be expressed in terms of the biomass of the juvenile sockeye salmon. A commonly observed inverse relationship between number of juvenile sockeye salmon and their average weight in sockeye salmon nursery lakes precludes the use of either of these parameters alone. Biomass is usually found by multiplying the average weight of the juvenile salmon with their estimated number. Until now, the estimation of numerical abundance has been an exceedingly more difficult task than that of assessing the average weight of the juvenile salmon, and the variance of the estimated biomass is therefore largely determined by the variance component due to estimation of numbers. With the advent of acoustical integrators, the accuracy of estimating numerical abundance may be increased decisively, necessitating a more precise estimate of the average weight of the juvenile salmon.

One problem encountered in weight estimation is related to the distribution of juvenile salmon. In Iliamna Lake, Bristol Bay, Alaska, which is about 90 miles long, the average weight of the juvenile salmon increases by about 20% from the upper (eastern) end of the lake to the lower (western) end. As a

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result the variance of an estimate of average weight of the salmon in the lake can be reduced by using a stratified sampling design. Further reduction of the variance is achieved by increasing the sample sizes. However, accurate weighing of the small salmon fry (age 0) is a difficult and time-consuming task. Weight data often do not exist, especially in older data, whereas length is commonly available. The problem of estimating weight from length data has recently been discussed by Pienaar and Ricker (1968). They assumed that the exponent to which length must be raised in order to give weight was constant. However, in the allometric relationship of weight and length, commonly accepted to be of the form

$$W = \alpha L^{\beta},$$

the exponent β reflects some of the yearly variability in feeding condition in the nursery area and the changing environmental conditions. In hatching operations the variable weight–length relationship is expressed in an alternate way by the so-called condition factor. When growth rates are compared a much higher precision is required than when biomass is studied. Thus, the two parameters α and β must be estimated, and they each possess their own sampling variance. Recently, Pienaar and Thomson (1969) examined two estimation procedures based on either a multiplicative or an additive error term. This note deals with another aspect by examining whether α and β most efficiently can be estimated from simultaneous observation of weight and length or whether the high correlation between these two variables can be used in the estimation process.

Materials and methods — Length–weight data were collected in Iliamna Lake over the years 1963–68. Two groups of juvenile salmon, fry (age 0) and yearlings (age 1), occur. They differ in average length by about 40 mm and in average weight by about 7.5 g. Samples were obtained from tow net catches, which were used to estimate the yearly abundance of juvenile salmon. Length was measured to the nearest millimeter and weight was taken to the nearest $\frac{1}{10}$ g, after the moisture had been wiped off with paper towels on the live specimens.

The same tow net schedule was followed each year, but the distribution of salmon shifted from year to year. As a result, the number of fish measured and weighed within each length and weight interval varied depending upon the distribution of the fish in a particular year, although most measurements clustered around the yearly means for length and weight of the two age-groups of juvenile salmon.

Analysis — The association between weight and length of fry and yearlings for the period 1963–68 was studied by a two-way analysis of covariance with age as a fixed factor and years as a random factor. The analyses were performed on the log–log transformed variables. The assumption that one common line could be fitted to all data was rejected (F = 15.20 with 22 and 3509 df). The less restrictive hypothesis of only a common slope was likewise rejected (F = 18.79 with 11 and 3509 df). Table 1 was constructed to decide if differences in slope and level were caused by either the age (a fixed factor with two levels) or the year (a random factor with six levels). In each case the F-tests gave the same results, and the large age–year interaction precludes

TABLE 1. Weight–length covariance analysis for juvenile sockeye salmon.

Source	Sum of squares	df	Mean square	F
		Common line		
Ages	.0102	2	.0051	.12
Years	.5790	10	.0579	18.86[a]
Interaction	.4311	10	.0431	13.91[a]
Error	10.7085	3509	.0031	
		Common slope		
Ages	−.0009	1	−.0009	.00
Years	.4179	5	.0836	27.23[a]
Interaction	.2163	5	.0433	14.09[a]
Error	10.7085	3509	.0031	

[a] Significant at the .05 level.

a direct interpretation of the effect of the main factors. However, when the intercepts of the 12 individual regression lines were plotted against the corresponding slopes a well-defined negative, linear relationship appeared except for the 1968 yearling observation. This small sample of fish of nearly same length contributed substantially to the total variability (Table 2).

Although the differences in slope and intercept may be significantly different from a statistical viewpoint, the actual differences encountered over the normal size range of fry and yearlings may be, at times, of minor practical importance and an average regression line can be used. For all data combined the equation has the form

$$W = .8168 \cdot 10^{-5} \cdot L^{3.027}.$$

A comparison of observed and computed weights for the two age-groups over the years 1963–68 is given in Table 2. The deviations of the weights computed from a common weight–length relationship and those based on yearly observations vary from year to year. The percentage deviations are higher for the fry than for the yearlings, which undoubtedly is related to the greater inaccuracies in weighing the small fry compared with the larger yearlings.

In the field where a crew must be maintained all the time, the cost of weighing a fish or measuring its length must be measured in time. The first operation takes about four times longer than the latter one. Furthermore, weighing cannot be done at times in rough weather, and is always less accurate than length measurement. On the average, the time ratio involved in weight–length measurements and length measurements alone under all weather conditions can be set at about 5:1; therefore, the time function to be minimized is

$$T = n \cdot C_n + n' \cdot C_{n'} \qquad (1)$$

TABLE 2. Average weight computed from yearly and combined weight–length data and variance estimates of juvenile sockeye salmon in Iliamna Lake, 1963–68.

Age-group	Year	$\alpha \cdot 10^{-5}$	β	Observed avg length (*mm*)	Computed avg wt (*g*)		Difference (%)	No. observations	$s_{y \cdot x}^2 \cdot 10^2$	$s_y^2 \cdot 10^2$
					Yearly parameters	Common parameters				
Fry	1963	.317	3.264	55.0	1.51	1.52	+.4	378	.803	7.811
	1964	3.653	2.656	45.5	.85	.92	+7.7	130	.478	3.315
	1965	1.716	2.844	55.7	1.57	1.58	+.8	191	.354	3.946
	1966	.450	3.168	52.2	1.30	1.25	−4.0	457	.331	8.605
	1967	2.221	2.945	52.5	1.32	1.33	+1.0	453	.492	8.516
	1968	2.965	2.707	57.5	1.73	1.72	−.7	331	.227	2.874
Avg fry								323	.448	5.844
Yearlings	1963	2.167	2.815	99.0	8.96	8.96	.0	205	.183	1.302
	1964	1.192	2.938	88.1	6.32	6.20	−1.9	463	.105	3.195
	1965	.56	3.139	97.1	8.45	8.72	+3.1	245	.123	2.418
	1966	.615	3.093	97.9	8.68	8.84	+1.8	85	.098	5.355
	1967	.476	3.149	85.9	5.83	5.85	+.2	555	.106	4.116
	1968	45.38	2.141	90.8	6.92	7.06	+1.6	40	.325	1.804
Avg yearlings								266	.157	3.032

where n is the number of weight–length measurements and n' the number of length measurements taken in a year. C_n is the time needed to weigh one fish and take its length, and $C_{n'}$ is the time needed to measure the length of one fish. It is assumed that n is fairly large, at least 100, and n' is larger. The two samples are assumed to be random samples.

The following exposition follows that given by Cochran (1964, p. 334–339). The variance of a weight estimate based on double sampling with regression is advantageous if

$$\rho^2 > \frac{4C_n \cdot C_{n'}}{(C_n + C_{n'})^2} .$$

Since values of the estimated correlation coefficients between the log–log transformed variables are extremely high, $r = .99$ for all data combined, $r = .96$ for fry alone, $r = .98$ for yearlings alone, $C_{n'} = 5$, and $C_n = 1$, the estimate of average weight is decisively improved by a double sampling with regression for a fixed amount of time available for weighing and length measurements. Even if weighing should require only twice as long a time as a single length measurement, the double sampling scheme is advantageous. The variance estimate of the average weight is

$$\hat{V}(\bar{y}_{lr}) = \frac{s_{y\cdot x}^2}{n} + \frac{s_y^2 - s_{y\cdot x}^2}{n'} . \tag{2}$$

In order to obtain a general allocation of n and n', equations 1 and 2 must be minimized simultaneously. This procedure leads to

$$\frac{n'}{n} = \sqrt{\frac{(s_y^2 - s_{y\cdot x}^2)\, C_n}{s_{y\cdot x}^2 \cdot C_n'}} . \tag{3}$$

As before, $C_n = 5$ and $C_{n'} = 1$ time units. The estimates of $s_{y\cdot x}^2$ and s_y^2 were taken as the average values obtained each year from 1963 to 1968. Fry and yearlings were kept separately (Table 2).

In the case of fry, the ratio between n and n' is obtained by substituting the appropriate values in equation 3.

$$\frac{n'}{n} = \sqrt{\frac{5.396 \cdot 10^{-2} \cdot 5}{.448 \cdot 10^{-2} \cdot 1}} = 7.8$$

or about eight length measurements for each weight–length measurement. For yearlings, the ratio is

$$\frac{n'}{n} = \sqrt{\frac{2.875 \cdot 10^{-2} \cdot 5}{.157 \cdot 10^{-2} \cdot 1}} = 9.6$$

or about 10 length measurements for each set of weight–length measurements taken. If the effort expended each year in the future were the same as that expended in the past, on the average 323 weight–length measurements of fry each year would correspond to 323 · (4 + 1) = 1615 time units. Then,

$$n \cdot (4 + 1) + n' \cdot 1 = 1615$$
$$8n = n'.$$

Consequently, $n = 124$ weight–length measurements and $n' = 994$ length measurements represent the optimal division of these two types of observations. For the years 1963–68, an average of 266 length–weight measurements of yearlings was taken; thus 266 . (4 + 1) = 1330 time units were expended.

$$n \cdot (4 + 1) + n' \cdot 1 = 1330$$
$$10n = n'.$$

Therefore, $n = 89$ weight–length measurements and $n' = 887$ length measurements is the optimal division of effort. By decreasing or increasing n and n' while maintaining the given ratios, one can give variance estimates to meet any desired precision.

Although the results derived are based on data from Iliamna Lake, the procedure is valid for all other sockeye lakes in Bristol Bay and probably elsewhere. At least the data presented should serve as a useful guidance for any future sampling program for a determination of the average weight of fry and yearling sockeye salmon once the desired degree of accuracy is specified.

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Temperature Conditions in the Gulf of Maine and Adjacent Waters During 1968

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COLTON, JOHN B., JR. 1969. Temperature conditions in the Gulf of Maine and adjacent waters during 1968. J. Fish. Res. Bd. Canada 26: 2746–2751.

Surface-water temperatures at Boothbay Harbor, Maine, in 1968 were appreciably higher than in 1967. This check in the recent coastal cooling trend was reflected in offshore surface and subsurface temperature conditions and was accompanied by a shift in the relative position of Coastal and Slope Water along the edge of the Continental Shelf.

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SINCE THE EARLY 1950s, a cooling trend in sea-surface temperature has been observed at Boothbay Harbor, Maine (Fig. 1), and at other locations along

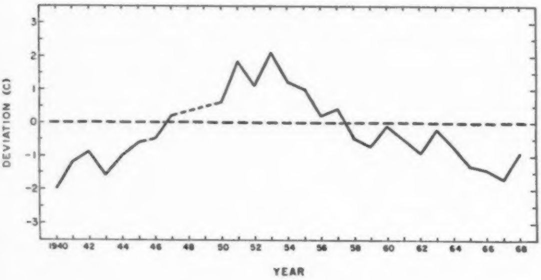

FIG. 1. Annual deviations from the 1940–59 mean sea-surface temperature at Boothbay Harbor, Maine. The base period 1940–59 was chosen because it included approximately equal numbers of years in periods of warming and cooling.

the coast of New England and the Maritime Provinces (Lauzier, 1965; Sterns, 1965; Welch, 1967). Professor H. C. Willet of Massachusetts Institute of Technology (personal communication) has predicted on a basis of long-term solar-climatic relations that this coastal cooling trend should terminate in the late 1960s. Thus, it was of interest to learn from Mr W. R. Welch of the Bureau of Commercial Fisheries, Biological Laboratory, Boothbay Harbor, Maine, that surface-water temperatures at Boothbay Harbor were appreciably higher during 1968 than 1967 (Fig. 2).

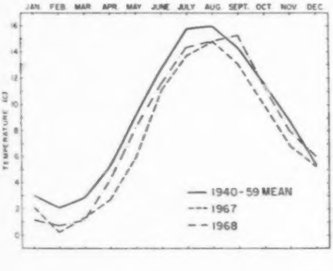

FIG. 2. Seasonal temperature curves, Boothbay Harbor, Maine, 1940–59, 1967, and 1968.

It has previously been shown that surface temperatures at Boothbay Harbor are a good index to offshore surface and subsurface temperature conditions in the Gulf of Maine and contiguous waters (Colton, 1968a). In addition, inshore and offshore temperature conditions both appear to depend in large measure on the relative position of coastal and oceanic water masses along the edge of the Continental Shelf. Years are warm when Slope Water borders upon the 200-m isobath and cold when Slope Water is displaced or modified by Coastal Water of Labrador origin (Colton, 1968b).

In this note offshore hydrographic conditions during September 1968 are compared with conditions in September 1965 and 1966 (Fig. 3) to determine if the offshore temperature anomalies reflect those observed at Boothbay Harbor and if there has been a corresponding shift in the distribution of Coastal

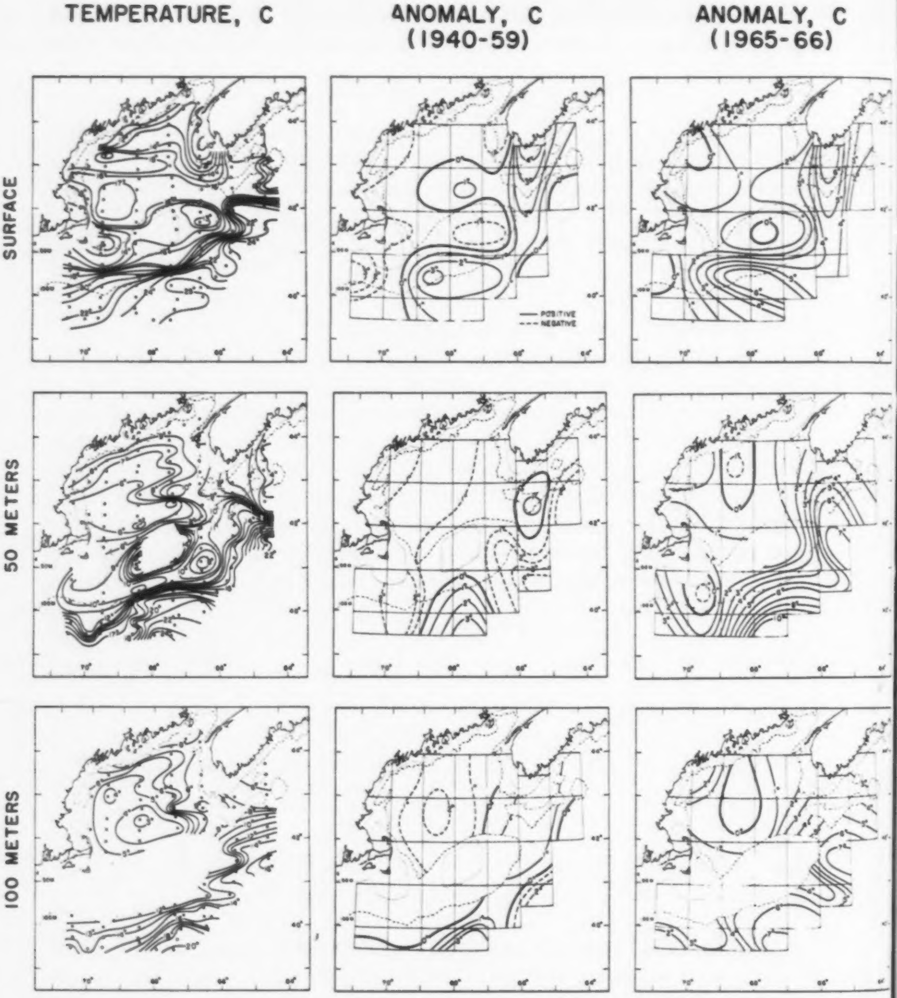
TEMPERATURE, C
ANOMALY, C (1940-59)
ANOMALY, C (1965-66)
SURFACE
50 METERS
100 METERS
POSITIVE
NEGATIVE
70°
68°
66°
64°

FIG. 3. Temperature distribution and temperature anomalies at the surface, 50 m, and 100 m duri September 1968 relative to the September 1940–59 and 1965–66 means. The anomalies were compute as the difference between the 1968 mean temperature and the 1940–59 and 1965–66 mean temperature within 30-min quadrangles.

and Slope Water along the edge of the Continental Shelf. With but few exceptions, the 1968 temperature anomalies were positive relative to the 1965–66 means at all three depths. These anomalies tended to increase with distance from shore and in a southeasterly direction. The 1968 temperatures were lower than the 1940–59 mean temperatures at all depths in the western Gulf of Maine and south of Cape Cod and at 50 m and 100 m in the southeast portion of the sampling area. The greatest positive anomalies were south of Nova Scotia and Georges Bank.

If we consider a temperature of 9 C and a salinity of 35‰ as roughly defining the northern limit of Slope Water (Worthington, 1964; Colton, 1968b), the Slope Water boundary coincided with the 200-m isobath in 1968 and extended appreciably north and east of its position in 1965 and 1966 (Fig. 4, 5). The subsurface dissolved oxygen concentrations corresponded to those typical of Coastal Water in 1965 and 1966 and to those typical of Slope Water in 1968 (McLellan, 1957). The temperature–salinity values (Fig. 6) were adjacent to the Slope Water curve in September 1968 whereas the values in this same area in September 1965 and 1966 were characteristic of Coastal Water (Colton, 1968b, fig. 7).

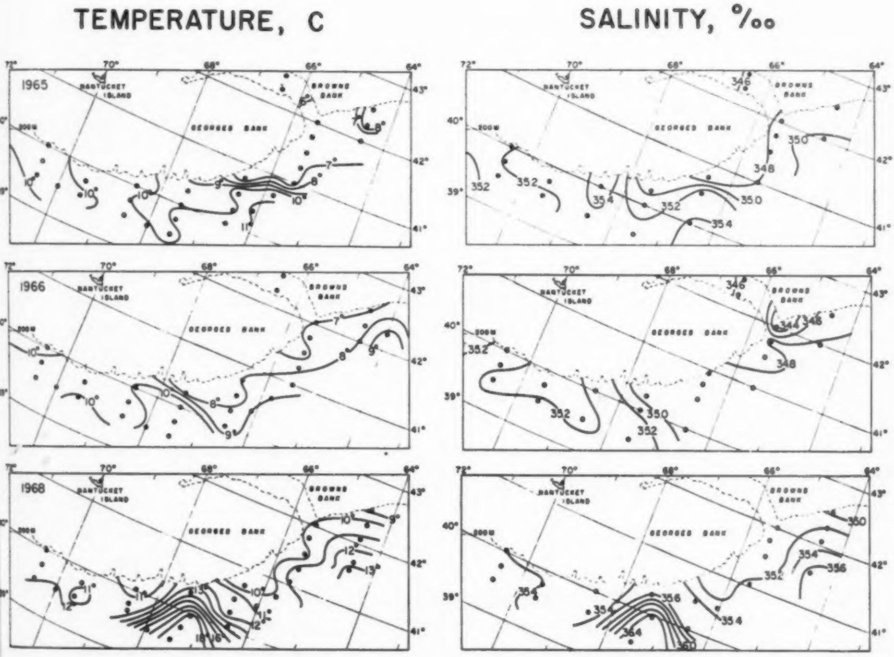
TEMPERATURE, C
SALINITY, ‰
1965
1966
1968
72°
70°
68°
66°
64°
43°
42°
41°
40°
39°
NANTUCKET ISLAND
BROWNS BANK
GEORGES BANK
200M
10°
9°
8°
7°
11°
12°
13°
16°
18°
352
354
350
348
346
344
356
360
364

FIG. 4. Temperature and salinity at 200 m during September 1965, 1966, and 1968.

Hydrographic conditions in September 1968 confirm previous conclusions in that the offshore temperature anomalies paralleled those observed at Boothbay Harbor and that the presence of Slope Water along the edge of the Continental Shelf coincided with a warming of surface and subsurface waters in the Gulf of Maine area. We plan to continue to monitor hydrographic conditions

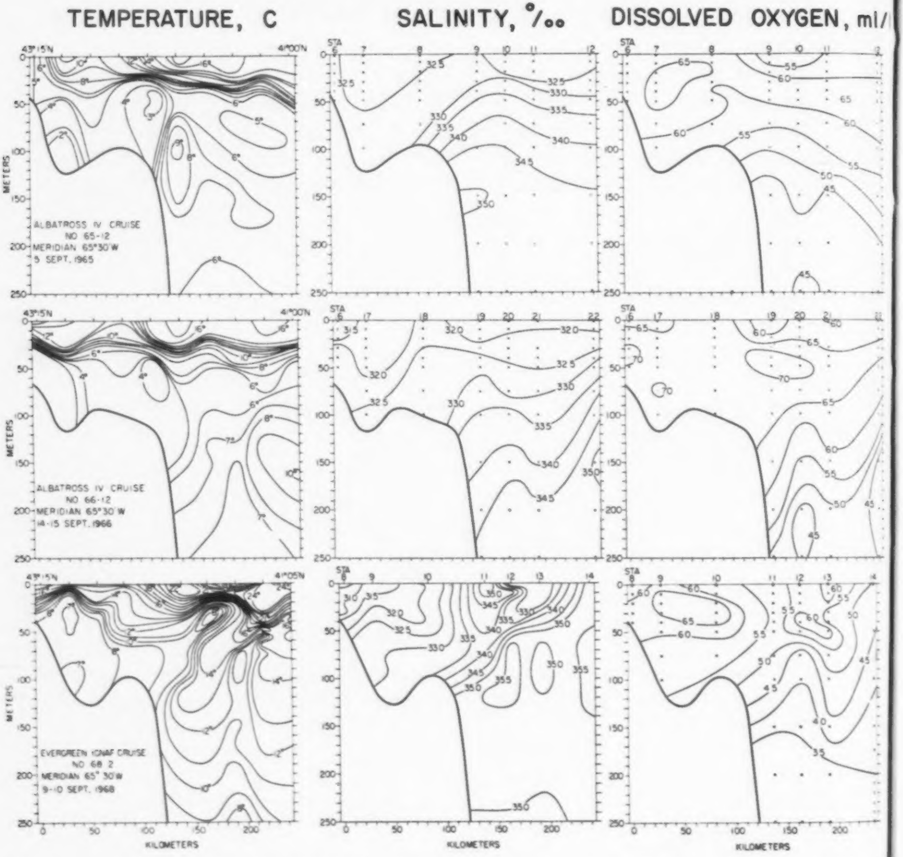

Fig. 5. Temperature, salinity, and dissolved oxygen profiles, meridian 65°30'W, September 1965, 1966, and 1968.

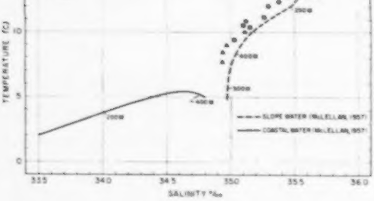

Fig. 6. Temperature–salinity relations at Evergreen stations 11–14, September 1968. The approximate depths in meters of waters of various temperature–salinity characteristics are indicated by numbers adjacent to the curves.

in critical areas along the Continental Slope to determine whether this reversal of the recent cooling trend is temporary or the beginning of a long-term warming trend.

The data were collected during *Albatross IV* Cruise 65-12, September 4–16, 1965, *Albatross IV* Cruise 66-12, September 8–23, 1966, and *Evergreen* ICNAF Cruise 68-2, September 8–17, 1968. The *Evergreen* data were made available by Mr M. Light of the U.S. Coast Guard Oceanographic Unit.

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Trawl-Board Sediment Sampler

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BUTLER, T. H., AND R. W. SHELDON. 1969. Trawl-board sediment sampler. J. Fish. Res. Bd. Canada 26: 2751–2753.

The design and operation of an underway sediment sampler are described, with particular reference to exploratory shrimp fishing. The sampler is fitted onto the shoe of a trawl otter board and automatically collects a bottom sediment sample at the beginning of a trawl tow.

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DURING 1966 AND 1967, exploratory surveys for new shrimp grounds were made on the continental shelf off British Columbia (Heritage and Butler,

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MS, 1967; Smith and Butler, MS, 1968). The most common commercial species is the smooth pink shrimp (*Pandalus jordani* Rathbun), and in other areas this species is known to occur most abundantly on a fine green mud bottom (Dahlstrom, 1961, 1969). Therefore, in addition to trawl samples, bottom sediment samples were also taken during the surveys. In 1966 the sediment samples were taken after each trawl tow with a Dietz-LaFond grab, but this was a time-consuming procedure. In 1967 a device was made to fit on the otter board to collect a sediment sample while the net was fishing. Considerable time was saved by combining the operations of shrimp and sediment sampling.

The sampling device consists simply of a short piece of steel pipe fixed at an angle of about 45° above an oval hole in the shoe of the trawl board (Fig. 1). The board measured 8 × 3⅓ ft (2.5 × 1.0 m), but the device could be attached to a board of any size providing it had a sufficiently wide shoe. As the otter board moves across the seabed, sediment is forced into the hole and is retained in the pipe.

The pipe consists of two parts (Fig. 2). The forward part is welded to the shoe about 2½ ft (0.75 m) from the leading edge, and the after part is attached to this by a bayonet fitting. The after end of the tube is sealed by a cap with a gauze-covered hole. This hole permits water to escape as the tube fills with sediment, but does not allow any significant amount of sediment to pass. Without this hole the sampler would not fill with sediment. At the end of each trawl tow the otter board is hauled onto the gallows until the lower edge is at gunwale height. The sample tube is then removed from the board and the sediment is taken out.

The sampler filled with sediment during the first 5 min of a tow. This is equivalent to a distance of about 220 yards (200 m). It is probable that on many occasions the sampler filled soon after the first impact of the trawl board with the bottom. It retained not only fine mud (shrimp ground) but also coarse sand and gravel (to a particle size of about 2.5 cm), although because of the small size of the opening it was probably not sampling the

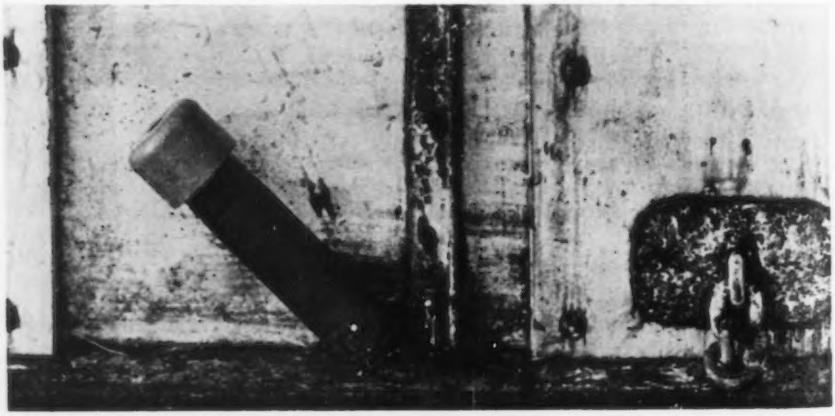

FIG. 1. The trawl-board sediment sampler.

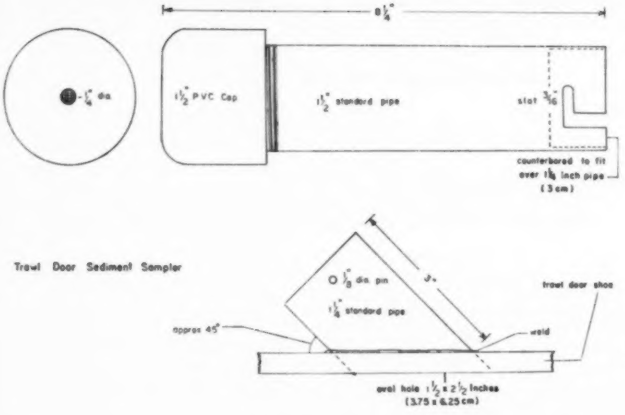
8¼"
¼" dia
1½" PVC Cap
1½" standard pipe
slot 3/16"
counterbored to fit
over 1⅛ inch pipe
(3 cm)
Trawl Door Sediment Sampler
⅛" dia. pin
1¼" standard pipe
3"
trawl door shoe
approx 45°
weld
oval hole 1½ x 2½ inches
(3.75 x 6.25 cm)

FIG. 2. Constructional details of trawl-board sediment sampler.

gravel quantitatively. In a series of initial tests, samples were taken from the same location with the trawl-board sampler and a Dietz-LaFond grab. There were no significant differences between the two sets of samples.

This device clearly provides a rapid and trouble-free method for simultaneous collection of sediment and biological samples, and could easily be adapted for a variety of tasks (e.g., deep-sea trawling). However, in order to retain coarse-grained sediment quantitatively, it should be made a little larger and the removable part of the pipe should be curved so that the end either lies horizontally or slopes downwards.

Acknowledgments — Construction and testing were made possible through funds given by the Industrial Development Service of the Department of Fisheries and Forestry of Canada. Mr K. Sutherland constructed the sampler and also helped with the design; Messrs M. S. Smith and J. C. Scrivener made practical tests during exploratory fishing surveys; and Mr A. N. Yates prepared the line drawing.

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Age and Size of Steelhead Trout in the Babine River, British Columbia

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NARVER, DAVID W. 1969. Age and size of steelhead trout in the Babine River, British Columbia. J. Fish. Res. Bd. Canada 26: 2754–2760.

Scales were collected from adult summer steelhead trout taken in 1967 and 1968 in an increasingly important sport fishery on the Babine River. The most common total age was 3.2 (73% in 1967 and 60% in 1968), and the second most common age was 3.3. The males tended to remain a year longer in the ocean than did females before first maturation. Four of 121 (3.3%) scales, all from female steelhead, indicated the fish had spawned once previously. The male to female ratio in the sport fishery was 1:1.33. Mean length at time of smolt outmigration as estimated from scales from 3.2 fish was 190 mm. These results are compared with what is known for other British Columbia steelhead stocks.

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STEELHEAD TROUT (*Salmo gairdneri*) in the Babine River are being increasingly utilized by a fall and winter sport fishery. Virtually nothing is known about the life history of this stock, and consequently there is no sound basis for its management. Presumably this is a "summer" run stock (as defined by Withler, 1966) since adult steelhead are commonly taken in salmon gillnets at the mouth of the Skeena River from late June to September. Furthermore, steelhead annually pass upstream through the counting fence located about 1.2 km below Nilkitkwa Lake (Fig. 1), beginning in late August and increasing in numbers through September or until the fence is removed in the fall (Aro, MS, 1961; Jordan, MS, 1967). Presumably this is only the early portion of

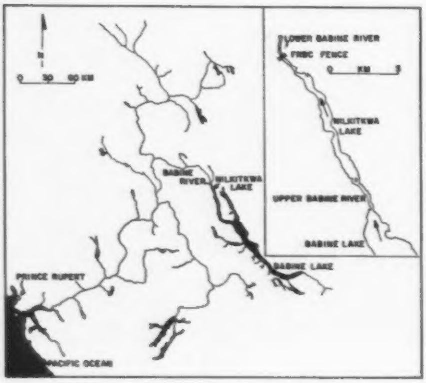

FIG. 1. Skeena River system and the outlet of Babine Lake.

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the population; thus the magnitude of the total run is unknown. The estimated sport catch of steelhead at Babine was 1640 fish in 1966–67 (British Columbia Fish and Wildlife Branch, MS, 1968) and 475 in 1967–68 (Petersen and Thomas, MS, 1969). (The 1966–67 figure is total catch, only about one out of three of which were killed (R. C. Thomas, B. C. Fish and Wildlife Branch, personal communication); the 1967–68 figure is only for fish killed.) Fisheries Research Board personnel have observed steelhead spawning in March, April, and May in the Babine River between the fence and the outlet of Nilkitkwa Lake, but not in the Babine River above Nilkitkwa Lake or in any of the tributaries of Babine Lake (Fig. 1). This note presents age and growth data obtained from scales taken from 121 maturing steelhead in the sport fishery during September and October 1967 and 1968 and compares these with similar data from other steelhead stocks in British Columbia.

Methods — Scales were taken from the fish within an area three scale rows above and below the lateral line and between the dorsal and anal fins. Scale impressions were made in cellulose acetate (Koo, 1962a). Measurement of the "lateral" (dorsal–ventral) diameter of the freshwater growth zones was at a magnification of 247 times, and ocean age was read at a magnification of 100 times. Ages of steelhead trout were established by comparing the independent analyses of two experienced scale readers. Those scales for which there was disagreement (about 27% of the total) were reexamined and a concensus reached. Spawning checks were expected to be well marked since Babine River steelhead spend many months in fresh water — from summer to the following April or May (Fig. 2).

Approximate length at time of formation of each freshwater annulus was obtained by treating the lateral diameter measurements of Babine scales with the relationship between lateral scale diameter and fish length for juvenile "winter" (as defined by Withler, 1966) steelhead originating in Chambers Creek, Washington (Vernon and McMynn, 1957). This relationship was derived from 96 steelhead that at 10 months of age ranged between 58 and 159 mm fork length. That juvenile lengths of Babine steelhead calculated from this relationship are only approximations can be appreciated because (1) all the Babine steelhead spend more than 1 year in fresh water, and thus smolts are presumably larger than 159 mm, i.e., beyond the size range from which the relationship is derived, and (2) possible differences in the scale diameter–fish length relationship may exist between Chambers Creek winter steelhead and Babine River "summer" steelhead. Furthermore, estimates of mean lengths at the time of formation of any freshwater annulus made from scales of adult steelhead in a particular year-class are probably overestimates because of better survival of the larger individuals (the reverse Rosa Lee Phenomenon of Chugunova, 1959).

The age designation used here is from a discussion of the European method of aging fish by Koo (1962b). A fish designated as 3.2+ spent three winters in fresh water before migrating to the ocean, where it spent two winters and part of a third summer before entering on its spawning migration, at which time it was captured. In this method spawning checks are counted as annuli. A 2.2+S1+ fish (Fig. 2) spent two winters in fresh water (2.) and two in the ocean (.2), returned to the river during its third summer or fall in the ocean (.2+), spent the winter in the river and spawned in the spring (S), emigrated from the river and spent that summer and winter in the ocean (1), and entered the river again in the following summer or fall (1+).

Weights of adult steelhead were measured with a spring scale.

Results and discussion — Of the steelhead trout scales collected in 1967 and 1968, 100 had a readable freshwater zone (scales from four fish that had spawned previously are excluded in this discussion). The most common total age (Table 1) was 3.2+ (73% in 1967, 51% in 1968), and the second most

TABLE 1. Summary of age compositions in numbers and percentages of maturing Babine River steelhead trout collected in 1967 and 1968. (Fish that had spawned previously are not included.)

	Total age										Freshwater age					Ocean age			
	2.1+	2.2+	3.1+	3.2+	3.3+	4.1+	4.2+	4.3+	5.1+	Total	2.	3.	4.	5.	Total	.1+	.2+	.3+	Total
1967																			
♂ (*n*)			1	11	3	2	1			18		15	3		18	4	17	6	27
%			5.5	61.0	16.7	11.1	5.5					83.3	16.7			14.8	63.0	22.2	
♀ (*n*)				24	2		2		1	29		26	2	1	29	2	29	2	33
%				80.0	6.7		6.7		3.3			89.7	6.9	3.4		6.1	87.9	6.1	
Total *n*			1	35	5	2	3		1	47		41	5	1	47	6	46	8	60
%			2.1	72.9	10.4	4.2	6.2		2.1			87.3	10.6	2.1		10.0	76.7	13.3	
1968																			
♂ (*n*)	1		1	9	8	1	1	1		22	1	18	3		22	3	11	11	25
%	4.5		4.5	41.0	36.5	4.5	4.5	4.5			4.5	81.8	13.6			12.0	44.0	44.0	
♀ (*n*)		1	1	18	4	2	5			31	1	23	7		31	3	25	4	32
%		3.2	3.2	58.1	12.9	6.5	16.1				3.2	74.3	22.6			9.4	78.1	12.5	
Total *n*	1	1	2	27	12	3	6	1	1	53	2	41	10		53	6	36	16	58
%	1.9	1.9	3.8	51.0	22.7	5.7	11.3	1.9			3.8	77.5	18.9			10.3	62.1	27.6	
Grand total	1	1	3	62	18	5	9	1	1	100	2	82	15	1	100	12	82	24	117
%	1.0	1.0	3.0	62.0	18.0	5.0	9.0	1.0	1.0		2.0	82.0	15.0	1.0		10.2	69.5	20.3	

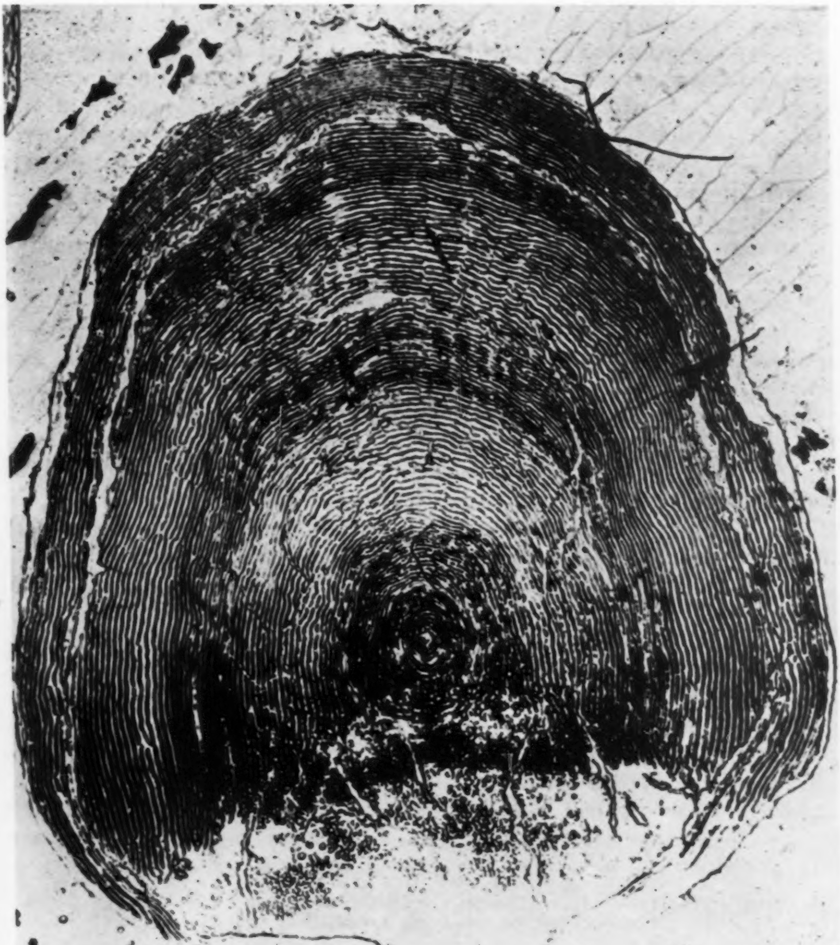

FIG. 2. Photomicrograph of a scale from a Babine River steelhead trout taken on September 21, 1967, showing a spawning check and having a scale formula of 2.2+S1+ (see text for explanation).

common was 3.3+ (10% in 1967, 23% in 1968). Over 87% of the sample in 1967 and 77% in 1968 had become smolts after spending three winters in fresh water. Of the 117 scales readable for ocean age, 77% in 1967 and 62% in 1968 were from .2+ fish. However, in both years, a markedly higher proportion of males than females occurred as .1+ and .3+ fish; a much higher proportion of females than males occurred as .2+ fish. Nearly all scales had some growth at the scale margin beyond the last ocean annulus that represented spring growth in the ocean prior to the assumed midsummer entry into the Skeena River. Resorption of the scale margin was not a problem in the interpretation of ocean age from these scales taken in September and October.

The age composition of Babine River steelhead, as derived from this sample, is more similar to that of summer steelhead from three streams in southern coastal British Columbia than to that of winter steelhead from eight streams in the same region. In the summer steelhead streams studied by Withler (1966) the most common total age was 3.2+, which occurred in 48–63% of the samples compared with 62% for Babine. Other fairly common ages for summer steelhead in the three southern British Columbia streams were 2.2+, 2.3+, and 3.3+ (Withler, 1966). In the Babine sample, one 2.2+ and no 2.3+ fish occurred, although 3.3+ was the second most common age. Of 50 steelhead scales taken in September and October 1961 in the Kispiox River, another tributary of the Skeena River, 31 were 3.2+ fish, and the next most common age-group (4.2+) consisted of 6 fish (G. D. Taylor, Fish and Wildlife Branch, personal communication).

The winter steelhead stocks discussed by Withler (1966) had the same four major age-groups as did the summer stocks but in proportions that varied widely among streams. The most common age-group in the winter steelhead stocks was 3.2 in five streams (34–49%), 2.2 in one stream (31%), and 3.3 in one stream (34%); 2.2 and 2.3 were equal in one stream (32%).

The percentages of 2. and .3 fish were generally much higher in steelhead streams from California to southern British Columbia (Withler, 1966) than for Babine River steelhead.

Distribution of weight by sex within the sample of 117 steelhead with readable ocean growth zones (exclusive of fish that had spawned previously) was closely related to ocean age (Table 2).

TABLE 2. Round weights (kg) of Babine River steelhead trout by ocean age-group and sex. (Fish that had spawned previously are not included.)

	♀			♂		
Ocean age	$\bar{x}$	Range	n	$\bar{x}$	Range	n
.1+	2.4	(1.4–3.4)	5	2.2	(1.5–3.2)	7
.2+	4.8	(2.7–6.3)	54	6.1	(3.6–8.2)	28
.3+	8.3	(6.6–10.0)	6	10.2	(8.2–12.6)	17

TABLE 3. Means and ranges of estimated fork lengths (mm) at time of formation of each freshwater annulus for Babine River steelhead trout taken in 1967 and 1968, according to age and year-class (calendar year of hatching) (sexes combined; second spawners are not included).

Year	Year-class	Age-group	*n*	1st year	2nd year	3rd year	4th year	5th year
1967	1963	3.1+	1	89	121	157		
	1962	3.2+	35	71(57–86)	121(92–200)	191(154–315)		
	1961	3.3+	5	68(55–77)	131(99–160)	208(138–272)		
	1962	4.1+	2	64(54–73)	93(76–110)	138(135–141)	192(183–203)	
	1961	4.2+	3	72(68–79)	113(102–120)	158(154–164)	226(194–267)	
	1961	5.1+	1	62	89	127	179	246
1968	1965	2.1+	1	70	124			
	1964	2.2+	1	71	137			
	1964	3.1+	2	66(59–73)	99(85–114)	200(152–248)		
	1963	3.2+	27	70(46–99)	121(80–147)	188(158–232)		
	1962	3.3+	12	64(54–75)	114(91–138)	180(134–214)		
	1963	4.1+	3	56(55–59)	86(75–93)	128(111–148)	201(177–215)	
	1962	4.2+	6	46(44–50)	83(74–94)	135(121–168)	194(173–252)	
	1961	4.3+	1	53	97	143	200	

Four of the 121 Babine River steelhead scales that were useable for ocean age (3.3%) indicated that the fish had spawned previously and were returning for the second time. These fish were all females, the age formulae, weights, and years of capture being as follows: 2.2+S1+, 6.5 kg (1967); 3.2+S1+, 6.8 kg (1968); 4.2+S1+, 8.2 kg (1968); 3.2+S+, 4.1 kg (1968). Note that in total age these fish could be designated as 2.4+, 3.4+, 4.4+, and 3.3+, respectively; but in terms of winters actually spent in the ocean they are .3+, .3+, .3+, and .2+, respectively, and in weight they correspond to those for these ages (Table 2). Three of these four fish apparently did not mature the winter following the first spawning but instead returned 2 years later. Little is known about the frequency of repeated spawning of steelhead. The proportion of previous spawners found at Babine (3.3%) is similar to that of second spawners in summer steelhead (5.0%) from lower mainland streams of British Columbia but is much lower than that (9.5–36.3%) for second spawners in winter steelhead (Withler, 1966).

The ratio of male to female steelhead in the sample of 121 scales with readable ocean age (including the four scales from previous spawners) from Babine River was 1:1.33 (from Table 1), and this ratio approximates that for other Pacific coast stocks of steelhead (Withler, 1966). Whether this reflects differential mortality and the true sex ratio of returning fish or selectivity in the sport fishery is unknown.

Approximate lengths at time of formation of freshwater annuli for the year-classes returning in 1967 and 1968 are summarized in Table 3. It is assumed that there is no difference in smolt length by sex, as it has been demonstrated that in fresh water both sexes of winter steelhead "grow at approximately the same rate" (Parker and Larkin, 1959). Smolts in the most important age-group (3.2+) had a mean length of about 190 mm (191 mm in 1967 and 188 mm in 1968). Those age-groups represented by more than one fish had a range of mean smolt length of 180–226 mm. The overall mean smolt length for 4. fish was 202 mm and that for 3. fish was 190 mm. Although most of the age-groups are represented by very small numbers of fish, a decrease in estimated length with increasing smolt age is apparent in the first, second, and third year in both 1967 and 1968. Such an observation is not surprising in view of Elson's (1957) work on the importance of size on transformation to the smolt stage in Atlantic salmon. Parker and Larkin (1959) showed that slow-growing steelhead remained in fresh water an additional year before becoming smolts.

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Bacterial Flora of Some Algal Foods Used for Rearing Bivalve Larvae

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MURCHELANO, ROBERT A., AND CAROLYN BROWN. 1969. Bacterial flora of some algal foods used for rearing bivalve larvae. J. Fish. Res. Bd. Canada 26: 2760–2764.

The heterotrophic bacteria present in 15 mass cultures of six different genera of algae used for feeding larvae of the Atlantic oyster, *Crassostrea virginica*, and the hard clam, *Mercenaria mercenaria*, were isolated, identified, and their physiology studied. Of the 120 isolates obtained, 118 (98.3%) were asporogenous, gram-negative rods — types commonly found in marine waters. The majority of the isolates, 98 (81.6%), belonged to the genus *Pseudomonas*.

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THE USE OF HATCHERIES for rearing economically important species of bivalve molluscs (such as the Atlantic oyster, *Crassostrea virginica*, and the hard clam, *Mercenaria mercenaria*) has become more attractive because of the advances made in the laboratory culture of these animals (Loosanoff and Davis, 1963). Usually, shellfish hatcheries in which oysters or hard clams are reared are based on essentially the same procedures. The animals are induced to spawn by thermal stimulation, their gametes are pooled, and the developing larvae are fed various unicellular algae until they reach metamorphosis. After metamorphosis the juvenile animals are kept in tanks of circulating sea water and reared until they are large enough to survive in the estuarine environment.

The food requirements of a hatchery are considerable, and algae often must be mass cultured in large quantities under somewhat crude conditions. Bacterial contamination is one of the many problems in the mass culturing of algal foods. The algal cultures invariably become bacterized to some extent even though axenic starter cultures are used and numerous precautions taken to try to maintain an axenic culture. A nutritionally acceptable algal food for bivalve larvae can become unacceptable because of its bacterial flora. Whether algal foods become unacceptable for the larvae because of the numbers or kinds of bacteria they contain has yet to be determined. The metabolic activities of large bacterial populations in bacterized algal cultures may rapidly promote conditions inimicable to good larval growth. Further, the presence of bacteria able to produce soluble toxins (Guillard, 1959) or capable of cellular invasion (Tubiash et al., 1965) foretells eventual significant or total mortality of the larvae.

A major objective of the micropathologist working with cultures of bivalve larvae is the isolation, identification, and selective elimination of experimentally verified bacterial pathogens from the larval cultures. These are all time-consuming processes, however, and a hatchery must be assured of a routine, adequate supply of acceptable algal food. If the algal cultures are bacterized, then perhaps knowledge of the abundance and generic composition of the contaminant bacteria may aid in the selection of antibacterials to use on a prophylactic or therapeutic basis.

The algal mass culture unit of this laboratory consists of 16 5-gal carboys contained in a thermostatically controlled water bath and illuminated by overhead fluorescent tubes (Davis and Ukeles, 1961). Usually, the individual carboys, once inoculated, are not dismantled unless the algae are growing poorly or show other signs of deterioration. The algal cultures sampled in this study consisted of seven carboy cultures of *Monochrysis lutheri* (3, 13, 42, 90, 108, 149, and 180 days old), three of *Isochrysis galbana* (22, 67, and 97 days old), two of *Phaeodactylum tricornutum* (36 and 83 days old), and one each of *Dicrateria* sp. (56 days old), *Chlorella* sp. (24 days old), and *Tetraselmis maculata* (51 days old). Approximately 50 ml were withdrawn from each carboy and placed in a sterile dilution bottle. Appropriate dilutions were made and plated on an organic agar containing 0.1% yeast extract (Difco), 0.1% trypticase (BBL), 1.5% agar (Difco), and aged, Millipore-filtered (0.45 μ) sea water. After incubation at 18 C for 7 days the plates were examined and morphologically dissimilar colonies were isolated. Pure cultures of the bacteria isolated were then studied morphologically and physiologically. The scheme used for identification was that of Shewan et al. (1960); physiological procedures followed the guidelines established by the Society of American Bacteriologists (1957).

Although we intended to characterize the bacteria in the algal cultures quantitatively, as well as qualitatively, realistic bacterial densities were not obtained with our sampling methodology. Phase contrast microscopy revealed that many of the bacteria in the algal cultures were (1) epiphytic, (2) aggregated on detritus, or (3) aggregated on other bacteria. Bacterial aggregates transferred across dilution series caused counting errors of considerable magnitude. Recently we have found that partial dispersion of these clumps can be achieved with a vortex mixer. Even with this method, however, colony counts on plates prepared at higher dilutions may overestimate the actual bacterial density of the algal culture.

The 120 bacterial isolates obtained from the cultures were identified as *Pseudomonas*, 98 (81.6%), *Flavobacterium*, 8 (6.6%), *Achromobacter*, 7 (5.8%), *Vibrio*, 5 (4.1%), *Bacillus*, 1 (0.8%), and *Corynebacterium*, 1 (0.8%). In all carboy cultures the dominant bacteria isolated belonged to the genus *Pseudomonas* (57.1–100% of the flora isolated); however, as with any isolation protocol involving culturing, the composition of the medium and the temperature of incubation may bias the results. The bacterial genera isolated from the individual carboy cultures and their frequency of occurrence are recorded in Table 1. Berland et al. (1969) found that cultures of the algae *Asterionella japonica*, *Chaetoceros lauderi*, *Lauderia borealis*, *Leptocylindricus danicus*, *Phaeodactylum tricornutum*, and *Stichochrysis immobilis* contained bacteria belonging to the genera *Pseudomonas*, *Flavobacterium*, *Achromobacter*, *Vibrio*, *Agarbacterium*, *Xanthomonas*, *Micrococcus*, and *Staphylococcus*. *Pseudomonas*, *Flavobacterium*, and *Achromobacter* together accounted for 19 of their 25 isolates.

TABLE 1. Percentage composition, by genera, of the bacterial flora of 15 carboy cultures of algae.

Culture	Genus[a] (%)					
	P	F	A	V	B	C
Monochrysis lutheri						
2	100.0	–	–	–	–	–
5	62.5	12.5	25.0	–	–	–
6	83.3	16.7	–	–	–	–
8	70.0	20.0	10.0	–	–	–
11	100.0	–	–	–	–	–
12	100.0	–	–	–	–	–
15	71.4	–	–	14.3	–	14.3
Isochrysis galbana						
3	77.8	11.1	11.1	–	–	–
4	100.0	–	–	–	–	–
14	100.0	–	–	–	–	–
Phaeodactylum tricornutum						
9	88.8	–	11.1	–	–	–
13	75.0	12.5	–	12.5	–	–
Chlorella sp.	62.5	12.5	–	12.5	12.5	–
Dicrateria sp.	71.4	–	14.3	14.3	–	–
Tetraselmis maculata	88.9	11.1	–	–	–	–
All	81.6	6.6	5.8	4.1	0.8	0.8

[a] P = *Pseudomonas*; F = *Flavobacterium*; A = *Achromobacter*; V = *Vibrio*; B = *Bacillus*; C = *Corynebacterium*.

Physiologically the isolates were more lipolytic (55.8%) than proteolytic (41.7%) or amylolytic (27.5%). Isolates that reduced nitrate to nitrite (55.0%)

and produced ammonia from peptone broth (14.1%) were more numerous than those that produced hydrogen sulfide from sodium thiosulfate (7.5%) and indole from tryptophan (3.3%). Approximately one-half of the isolates (51.6%) grew in a basal medium of sea water enriched with ammonium chloride and glucose. Tests of the ability of the isolates to grow in seawater broths at salt concentrations of 0.5, 1.0, 1.5, 2.0, and 2.5% showed that 57.5%, 73.4%, 80.0%, 85.0%, and 87.4%, respectively, were able to grow. Lawn and disc sensitivities revealed that 91.6% of the isolates were sensitive to chloramphenicol (30 μg), 37.5% to penicillin (10 units), 65.0% to streptomycin (10 μg), and 75.0% to tetracycline (30 μg). All the isolates were able to grow at 37 C. A summary of physiological reactions for the isolates appears in Table 2.

TABLE 2. Biochemical activities of bacteria isolated from 15 carboy cultures of algae.

	% active			
Activity	*Pseudomonas*	*Flavobacterium*	*Achromobacter*	All[a]
Amylase	26.8	12.5	–	27.5
Gelatinase	37.2	12.5	28.6	41.7
Lipase	65.0	12.5	14.3	55.8
Nitrate reduction	60.8	–	14.3	55.0
Ammonia from peptone	13.4	–	–	14.1
Production of hydrogen sulfide	4.1	25.0	28.6	7.5
Indole production	–	–	–	3.3
Growth in basal medium	47.4	62.5	71.5	51.6
Growth in medium with:				
0.5% NaCl	60.8	50.0	28.6	57.5
1.0% NaCl	77.4	37.5	42.9	73.4
1.5% NaCl	84.5	37.5	42.9	80.0
2.0% NaCl	89.7	50.0	57.2	85.0
2.5% NaCl	92.8	75.0	57.2	87.4
Sensitivity to:				
Chloramphenicol	90.8	87.5	100.0	91.6
Penicillin	30.9	75.0	57.2	37.5
Streptomycin	67.0	25.0	57.2	65.0
Tetracycline	74.2	87.5	71.5	75.0
Growth at 37 C	99.0	100.0	100.0	99.1

[a] Including *Vibrio*, *Bacillus*, and *Corynebacterium*.

Evidence grows that bacteria are implicated in the mass mortalities observed in cultures of bivalve larvae. Although considerable effort is made to maintain the mass cultures of algae used as foods essentially free from bacteria, this objective is rarely accomplished. All carboy cultures examined were heavily bacterized, irrespective of whether they were young or old cultures. The heterotrophic bacterial flora isolated was more typically aquatic than aerial. Whether starter cultures are not truly axenic or whether contaminants are introduced while the unit is in use was not determined. The effects on the larval cultures of the repeated additions of bacterized foods will vary, depending on the bacteria introduced. If a particular bacterized algal culture is unacceptable as a larval food, one can either discard the culture, use antibacterials therapeutically in the algal culture, or use antibacterials prophylactically in the larval culture. The most effective use of antibacterials can be made only after the composition of the bacterial flora is determined. Since the isolation and sensitivity testing of bacteria in the algal cultures is time consuming, it may be more expedient to administer broad-spectrum antibacterials without delay. Recent experiments in our laboratory have shown that many initially unacceptable algal foods can be made acceptable for oyster larvae by supplementing them with various antibacterials. Algal foods that are acceptable without antibacterials do not become appreciably better foods with the addition of antibacterials. Studies of the heterotrophic bacterial flora commonly associated with the mass cultures of algae might reveal more effective means of suppressing their activities in bivalve larval cultures.

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Rapid Cold-Branding of Salmon and Trout with Liquid Nitrogen

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MIGHELL, JAMES L. 1969. Rapid cold-branding of salmon and trout with liquid nitrogen. J. Fish. Res. Bd. Canada 26: 2765–2769.

Liquid nitrogen (at −196 C) was successfully used as a coolant in a simple, inexpensive, and rapid method of marking salmon and trout with a metal branding tool. The marks, applied to as many as 650 fish per hour per marker, were consistently clear and readily identifiable for at least 14 months after application. No mortalities could be attributed to the branding technique among 4000 yearling coho salmon held for 2 months after marking.

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THIS NOTE DESCRIBES A METHOD by which liquid nitrogen at −196 C was used as a coolant in a simple technique for cold-brand marking large numbers of juvenile salmon and trout rapidly and continuously. In this method the fish were marked by placing them against a stationary cold-branding tool cooled by an attached reservoir of liquid nitrogen, rather than by placing a hand held tool against the fish as in previously described thermal-branding techniques.

Fujihara and Nakatani (1967) successfully cold-branded fish for the first time with metal tools chilled by a slurry of dry ice and ethanol (−78 C). However, my attempts to mark large numbers of fish rapidly and continuously were unsuccessful when dry ice and ethanol were used. Ice and frozen mucus accumulated on the branding tool and apparently disrupted heat transfer between the fish and the tool. The resultant marks were poor. Good marks were produced when the tool was cleaned regularly but the rate of marking was greatly reduced. When liquid nitrogen was used the tool remained free of ice and mucus and produced consistently clear brands at rapid rates.

Materials and methods — Liquid nitrogen, a reservoir, and branding tools were all that were necessary. The reservoir used for the test marking was made by carving a cavity that was 3 inches in diameter and 3 inches in depth (capacity 350 ml) from a piece of rigid polyurethane foam of 2-lb density. A 3-inch thickness of the insulation was left around the side and bottom of the cavity, and a lid 3-inches thick was placed loosely over the top. The branding tool (Groves and Novotny, 1965), with the plastic tubing removed, was inserted so that the handle was immersed in the liquid nitrogen and the branding surface was outside (Fig. 1A). A brass connector fitting for ¼-inch copper tubing, its internal ferrule replaced with adhesive tape, was cemented over the outside opening. This arrangement (Fig. 1B) facilitated changing tools and prevented leakage. Several tools could be mounted on one container to provide a choice of marks or permit simultaneous use of the reservoir by more than one operator.

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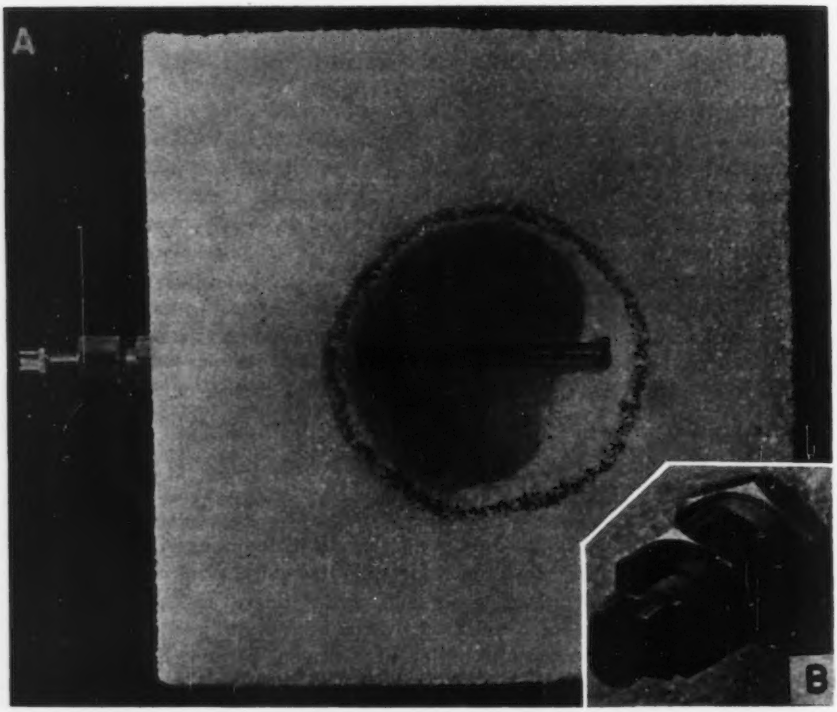

FIG. 1. (A) View from the top of the cold-branding apparatus; the lid was removed to show the reservoir for liquid nitrogen (outlined in black on the foam). (B) Close-up of male tubing connector and "U"-shaped cold-branding tool mounted for marking.

For marking, 350 ml of liquid nitrogen was placed in the reservoir. Within 3 min the tools were sufficiently cooled to begin marking. The fish were taken directly from an anesthetizing solution of M.S. 222 (tricaine methanesulfonate) to the stationary branding tool and marked by gently pressing the fish against the exposed branding surface (Fig. 2). The thumbs were used

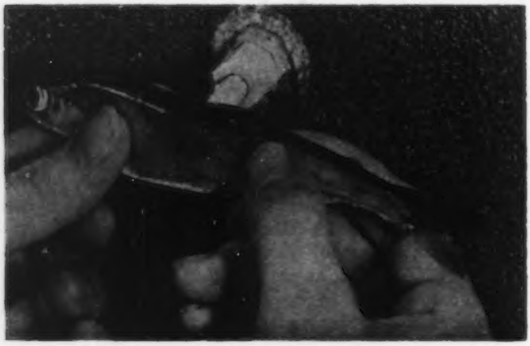

FIG. 2. Cold-brand marking a juvenile sockeye salmon with liquid nitrogen as the coolant.

to apply just enough pressure to indent the skin slightly at the point of tool contact. When fish were rolled across the tool, as opposed to steady pressure, there was a substantial reduction in clarity and preservation of the marks.

The fish were cold-branded with ½-, 1-, 2-, and 3-sec periods of skin-to-brand contact to determine if short exposure would effectively mark and to see if a long exposure (3 sec) might be harmful. Everest and Edmundson (1967) branded fish for 3 sec with tools chilled with dry ice and ethanol and experienced no adverse effects. Fujihara and Nakatani (1967) branded for 2 sec only.

Liquid nitrogen is available at costs ranging from $0.05 to $1.00 per liter, depending upon the supplier and the quantity purchased. The extremely low temperature and rapid evaporation (expansion) of liquid nitrogen make it imperative that the operator observe the following safety precautions: (1) always provide venting for the gaseous nitrogen formed by evaporation; (2) use protective clothing and goggles when drawing or pouring liquid nitrogen; and (3) anchor the reservoir securely during operation.

Usefulness of the technique — Juvenile chinook salmon, coho salmon, sockeye salmon, and steelhead trout ranging from 65 to 160 mm in fork length developed clear, well-defined marks. All the brand application times (½–3 sec) were equally capable of producing consistent, identifiable marks. For about 7 weeks the marks were-dark with sharp definition (Fig. 3A), after which they became of lighter hue and consisted mainly of altered scales and scarred epidermis (Fig. 3B).

Marks from each of the brand application times were identifiable in juvenile salmon for at least 8 weeks after marking but some mark loss was noted in groups branded for ½ sec when held for more than 8 weeks. Four thousand coho salmon (averaging 77 mm in fork length) were branded for 1 sec and held in an outside raceway for 2 months after marking. Their behavior was normal throughout the holding period, during which only five fish died, and the mark was still easily recognized at the end of the holding period. Eight sockeye salmon (averaging 80 mm in fork length) branded for 1 sec retained identifiable marks for 14 months, the period they were held. Fry (less than 55 mm in fork length) were marked, but the marks were not identifiable a short time later (approx 3 weeks).

More work is needed to determine the optimum duration of brand application to the epidermis of the fish and the best size and age at which the brands should be applied to ensure identifiable development and maximum retention. However, the limited data now available suggest that fish should be over 55 mm in fork length and should be marked for at least 1 sec.

About 40,000 salmon and trout have been branded by the liquid nitrogen method and released at the Seattle Biological Laboratory since the completion of initial tests in February 1967. The technique has also been field tested by the laboratory. Over 5,000,000 juvenile salmon and trout were marked using liquid nitrogen during the 1968 and 1969 downstream migrations on the Columbia River.

No mortalities or other harmful effects were attributable to use of liquid nitrogen in this technique at any of the brand application times (½–3 sec)

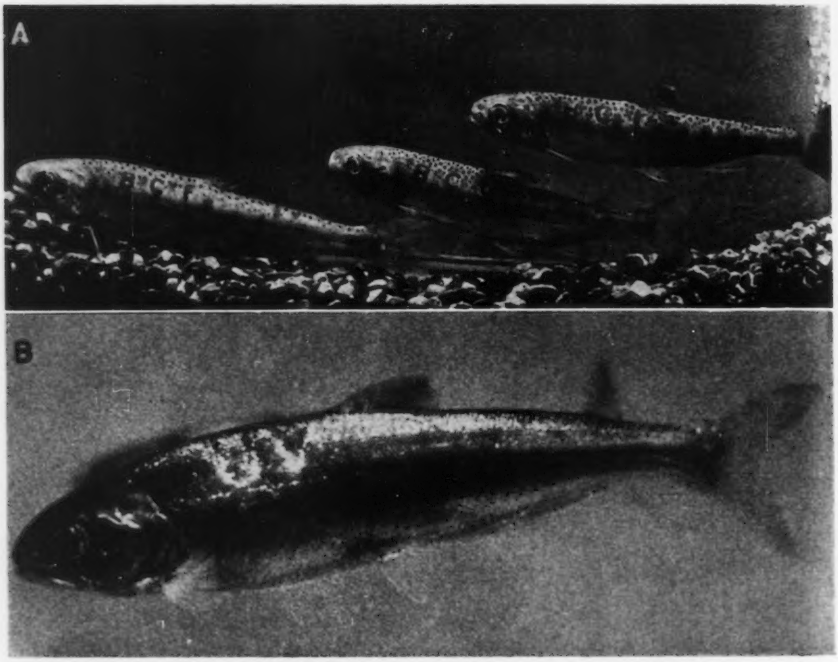

FIG. 3. (A) Cold-brands ("BCF") 48 hr after marking. The fish are yearling coho and sockeye salmon that average about 100 mm in fork length. (B) Twelve-month-old cold-brand ("UV") on a juvenile sockeye salmon, 230 mm in fork length. The fish was 80 mm in fork length when the ¼-inch letters were applied; the mark shown is ¾-inch high.

in either the laboratory or the field. Occasional mortalities could always be traced to overdoses of anesthetics or insufficient aeration in holding vessels.

Marking speed varied with different operators and with size of fish; smaller fish were more difficult to hold and consequently took longer to mark. Fish between 80 and 160 mm in fork length were easily marked at a rate of about 450 fish per hr per marker; with special effort the rate was increased to about 650 fish per hr.

The effective marking time that could be obtained from one filling of the liquid nitrogen reservoir (350 ml) depended upon the rate of evaporation, the number of tools mounted, and whether more than one operator used the reservoir at the same time. When only one tool was used, 350 ml of liquid nitrogen lasted for about 2 hr of continuous branding at a rate of 450 fish per hr. When two operators simultaneously used tools mounted on opposite sides of the reservoir, the coolant lasted about 1½ hr. The only effect of simultaneous marking by two operators appeared to be a more rapid use of liquid nitrogen, with no change in quality of the markings.

The cost of liquid nitrogen for 2 hr of branding (including consideration for evaporation during reservoir filling) was calculated at 3–40 cents. The low cost, coupled with the simplicity in the reservoir design, ease of marking, and effectiveness of the method at rates much more rapid than previously possible by other branding techniques, should make the method attractive to any agency wishing to mark large numbers of fish.

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First Records of *Ariomma bondi*, *Caranx crysos*, and *Selar crumenopthalmus* (Pisces) in the Gulf of St. Lawrence

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MACKAY, K. T., AND GETHIN THOMAS. 1969. First records of *Ariomma bondi*, *Caranx crysos*, and *Selar crumenopthalmus* (Pisces) in the Gulf of St. Lawrence. J. Fish. Res. Bd. Canada 26: 2769–2771.

The discovery of *Ariomma bondi* in the southern Gulf of St. Lawrence indicates a significant northward extension of its range. Specimens of *Caranx crysos* and *Selar crumenopthalmus* are the first records for the Gulf of St. Lawrence.

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IN SEPTEMBER 1967, the three species reported in this note were obtained amongst catches of Atlantic mackerel, *Scomber scombrus*, and white hake, *Urophycis tenuis*, taken from a trapnet set in 8 m of water, in Georges Bay off Cribbean Head, Antigonish County, Nova Scotia.

The lengths were taken as fork length (FL), from the tip of the snout to the tip of the central caudal rays, and total length (TL), from the tip of the snout to the tip of the caudal fin in its natural position. Meristic counts were obtained from radiographs.

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Our identifications of the carangids were confirmed by Dr W. B. Scott, Royal Ontario Museum, Toronto, Ont., who initially identified *Ariomma*, and this identification was confirmed by Dr R. L. Haedrich, Woods Hole Oceanographic Institution, Woods Hole, Massachusetts. The specimens with the exception of *Selar crumenopthalmus*, which was lost, are stored in the Royal Ontario Museum.

Ariomma bondi Fowler, 1930 (Suborder: Stromateoidei; Family: Ariommidae) — A single specimen (Fig. 1) of *Ariomma* was collected September 20, 1967 (Royal Ontario Museum Catalogue No. 26,577). This genus is divided into two groups, those with deep bodies and those with elongate bodies. The elongate body group, to which our specimen belongs, has two centers of distribution, one in the tropical and western North Atlantic and the other between Hawaii and Japan in the Pacific Ocean. Taxonomy at the species level in the Atlantic ariommids is in a state of flux but is currently under review by Haedrich. The specimen was originally identified as *Ariomma nigiargentea* (Ginsburg, 1954), a species that Haedrich (1968) reduces to the synonymy of *Ariomma bondi* Fowler, 1930.

The snout of our specimen was damaged so that the actual length measurements (135 mm FL, 150 mm TL) are underestimates of the true length. The radiograph (Fig. 1) shows the bony skeletal elements of our specimen, and the toothed outgrowths in the gullet that are typical of many of the stromateoids are very apparent. The vertebral number (31), the first dorsal fin ray count (11), the second dorsal fin ray count (15), and the anal fin soft rays (15) all conform to the range of these characters for *A. bondi* (Haedrich, 1967, 1968). The anal fin, however, had only one spinous ray in contrast with two or three, which are characteristic of *A. bondi*.

Since this species matures around 160–180 mm SL and does not grow much larger (Haedrich, 1967) our specimen is probably immature. Although very small specimens are pelagic, specimens comparable in size to ours are bottom feeders normally found at depths exceeding 100 m (Haedrich, 1967). Our specimen was captured in shoal water at a depth of 8 m in a bay with a maximum depth of 36 m, This specimen represents a significant northward range extension since it appears to be the first record of *A. bondi* north of Cape Cod, and thus is the first record of this species for Canadian waters.

Caranx crysos Mitchill, 1815; blue runner (Family: Carangidae) — A specimen of *Caranx crysos* was captured on September 8, 1967 (177 mm FL, 204 mm TL), and another on September 20, 1967 (168 mm FL, 196 mm TL, Royal Ontario Museum Catalogue No. 26,525). The trap operator, Mr V. E. Boudreau, reported seeing similar specimens in late August and early September, 1967, and also on two other occasions within the last 30 years.

The meristic counts of our specimens agree with the ranges for this species given by Leim and Scott (1966).

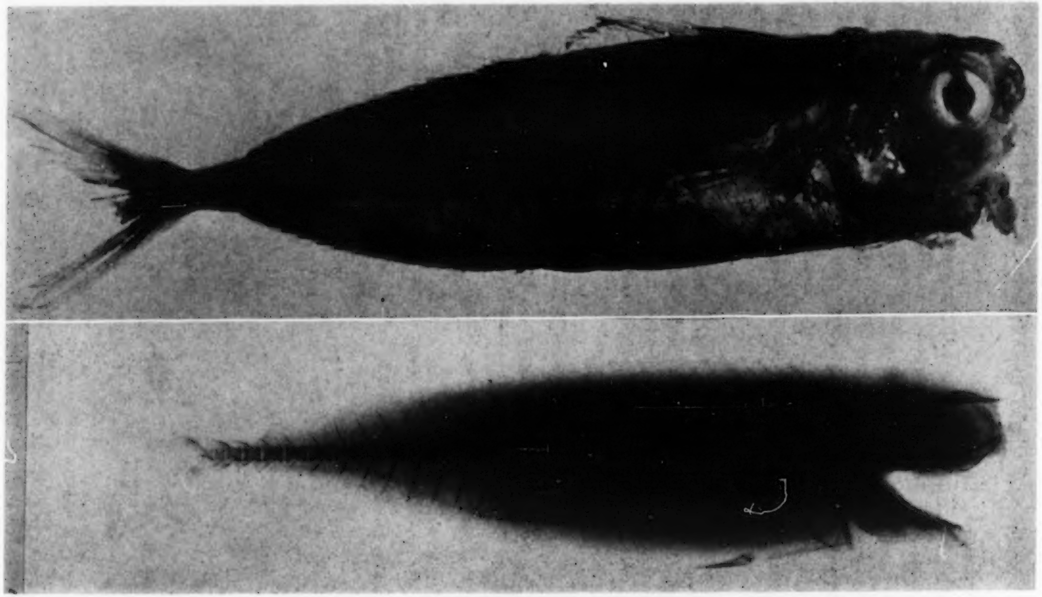

FIG. 1. *Ariomma bondi* collected September 20, 1967, from Cribbens Point, N.S.: *above*, photograph; *below*, radiograph.

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Although listing few records, Leim and Scott (1966) claim *C. crysos* occurs sparingly along the Atlantic coast of Nova Scotia. Our specimens appear to be the first record for the Gulf of St. Lawrence.

Selar crumenopthalmus Bloch, 1793; bigeye scad (Family: Carangidae) — A single specimen of *Selar crumenopthalmus* (133 mm FL, 150 mm TL) was collected on September 8, 1967. This specimen was lost in shipping but the radiograph was used by Dr W. B. Scott for verification of our preliminary identification. Leim and Scott (1966) report *S. crumenopthalmus* only twice in eastern Canadian waters. Both records are from the Atlantic coast of Nova Scotia and MacKay (unpublished data) has collected another specimen in St. Margarets Bay, N.S. Our specimen is the first record for the Gulf of St. Lawrence.

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